University of Alberta

mtDNA and Prehistoric Siberian Hunter-Gatherers: Characterising Matrilineal Population Affinities in Neolithic and Bronze Age Cis-Baikal

by

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Abstract

The evolution of the polymerase chain reaction (PCR) has been fundamental to the analysis of ancient DNA (aDNA) from human remains. Using aDNA polymorphisms retrieved from human skeletal material, it is possible to reconstruct the life history of an individual by revealing population origins, sex and disease polymorphisms, if present. This dissertation presents the aDNA analysis of two prehistoric cemetery populations from the Cis-Baikal region of Eastern Siberia which date from 6125 to 2220 B.C. These two cemeteries, known as Lokomotiv and Ust-Ida, flank a putative 800-year hiatus in the archaeological record of the Cis-Baikal region. Based on differences in cultural behaviour and osteological traits, the groups buried at Lokomotiv and Ust-Ida are believed to be biologically distinct. Using PCR primer sets targeting Asian-specific mitochondrial DNA (mtDNA) substitutions, the people at Lokomotiv and Ust-Ida were found to possess six different mtDNA haplogroups including A, C, D, F, G2a and U5a. The mtDNA haplogroup distributions for the Lokomotiv and Ust-Ida samples differ significantly, thus supporting the hypothesis that these groups were biologically distinct. Biological distance algorithms, used to compare the Lokomotiv and Ust-Ida mtDNA datasets with those for other Siberian and East Asian groups, showed the Lokomotiv and Ust-Ida samples to have different population affinities. While Lokomotiv was observed to cluster with modern Yenisei river basin groups to the west of Cis-Baikal, the Ust-Ida sample was most similar to groups living proximate to Cis-Baikal including a Northern Mongolian cemetery population dating to the 3rd century B.C. These differing mtDNA affinities suggest that the biological hiatus began with the migration of the Lokomotiv community from the Cis-Baikal region and ended when the group at Ust-Ida moved into the area. Furthermore, while the

descendents of the Lokomotiv people may have contributed to the subsequent matrilineal population structure of modern Yenesei river basin groups, descendents of the group at Ust-Ida likely remained in the Cis-Baikal region until the 2nd century A.D. By comparing the aDNA data for Lokomotiv and Ust-Ida against various lines of archaeological evidence, several associations between biological affinity and differential mortuary ritual were illuminated; these associations suggest that biological affinity influenced the type of grave one was buried in as well as where one was buried at either cemetery.

For my Grandmothers- Ena Mary Helen Driscoll and Harriet Dean Stead. Your collective wisdom and spirit have always and will continue to be my guide.

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Table of Contents

Chapter 1: Introduction 1

Literature Cited 8

Chapter 2: Ancient DNA Analysis of Human Cemetery Populations 10

The biology of DNA 11

mtDNA 11

Asian mtDNA variation 14

Nuclear markers for aDNA studies 18

The chemistry of ancient DNA 20

Hydrolysis 20

Oxidation 22

Characterising the postmortem persistence of DNA 23

Physiochemical properties of DNA in tissue 23

Environmental influences on aDNA preservation 25

Temperature 25

Water 26

pH and soil type 27

Radiation 27

Microorganism activity 28

Studies of taphonomic DNA decay 29

The manipulation of aDNA 30

    Ancient DNA analytical strategies 30
    Challenges to aDNA authenticity 32
    Contamination 34
    Establishing the authenticity of aDNA 37
Population genetics and the statistical interpretation of aDNA data 38
    Overview 38
    Inter-population comparisons 39
    Intra-population affinities 45
Ancient DNA applications 47
    Overview 47
    Biological distance Studies 49
    Biological affinities studies 52
Literature Cited 57

Chapter 3: Verifying the Authenticity of mtDNA data from Curated Human Skeletal Remains 75

Introduction 77
Materials and Methods 77
    Samples 77
    Specimen handling 78
    Specimen preparation 79
    DNA extraction 80
    PCR amplification and DNA sequencing 80
Results and Discussion 81

Summary 88

Literature Cited 90

Chapter 4: The Matrilineal Population Structure of Prehistoric Siberia: An Examination of Biological Continuity in Neolithic Cis-Baikal 93

Introduction 94

Materials and Methods 99

- Neolithic population samples 99
- Specimen preparation 101
- Contamination controls 101
- Extraction 102
- PCR amplification 102
- RFLP haplogroup assignment 104
- Evaluation of authenticity 105
- Statistical analysis 105

Results and Discussion 109

- Analytical success 109
- Neolithic mtDNA haplogroup assignments 109
- Biological distance of Kitoi and Serovo-Glazkovo 112
- Population affinities of Kitoi and Serovo-Glazkovo 116
- Kitoi population affinities 117
- Serovo-Glazkovo population affinities 122
- An ancient link between the Serovo-Glazkovo and the Huns? 125

Conclusions 129

Literature Cited 133

Chapter 5: A Tale of Two Cemeteries: Exploring Relationships Between Mortuary Ritual and Biological Affinities in Neolithic Lake Baikal Hunter-Gatherer Populations 141

Introduction 143

Materials and Methods 145

- Cemetery descriptions 145
- Samples 146
- Specimen preparation and DNA extraction 147
- mtDNA RFLP and HVI sequence analysis 148
- Molecular sexing 149
- Contamination control 150
- Evaluation of authenticity 151
- Statistical analysis 152

Results and Discussion 152

- Rationale 152
- Lokomotiv 153
- Ust-Ida 165

Conclusions 181

Literature Cited 186

Chapter 6: General Discussions and Conclusions 194

Literature Cited 205

Appendices 206

Appendix 1: Statistical software for ancient DNA population comparisons..207

Appendix 2a: Summarised mtDNA HVI sequences from deliberately handled samples 208

Appendix 2b: Raw sequence data for deliberately handled samples 210

Appendix 3: Primers for RFLP, HVI and amelogenin targets analysed in this study 220

Appendix 4: mtDNA haplogroup frequencies for modern and Neolithic Asian populations analysed in this study. 221

Appendix 5a: Summarised RFLP and HVI sequence data 222

Appendix 5b: Representative samples for RFLP analysis 227

Appendix 5c: Representative samples of aDNA HVI sequences.......................237

Appendix 6: Lokomotiv site map 258

Appendix 7: Ust-Ida site map 259

Appendix 8: Map of Sector 2 burial distribution..260

Appendix 9: Molecular sex results 261

Appendix 10: *Curriculum vitae* 265

List of Tables and Figures

Table 1-1: Culture history model for prehistoric Cis-Baikal hunter-gatherer cultures (adapted from Weber et al., 2002) 3

Table 2-1: Founding Asian mtDNA variants 16

Figure 2-1: Principal sites of hydrolytic attack on DNA 21

Figure 2-2: Deamination of cytosine to uracil 22

Figure 2-3: Oxidative conversion of guanine to 8-hydroxyguanine 23

Table 2-2: Allele frequencies for five hypothetical populations 42

Figure 2-4: Principal component map constructed from R-matrix computations for five hypothetical populations 42

Figure 2-5: Biological distances between five hypothetical populations computed from F_{ST} 43

Figure 2-6: PC map of F_{ST} correlation matrix between five hypothetical populations 44

Figure 4-1: Location of Neolithic Cis-Baikal cemeteries analysed in this study ... 96

Figure 4-2: Map of modern Siberian populations used for comparison in this study 107

Table 4-2: HVI sequences found in the Kitoi and Serovo-Glazkovo 111

Figure 4.3: Plot of mtDNA diversity against biological distance for prehistoric and modern Asian groups. 115

Table 4-3: Chronological distribution of Kitoi mtDNA haplogroups 116

Figure 4-4: PCA of R-matrix for prehistoric and modern Asian mtDNA Data Key for population groups: 118

Figure 4-5: F_{ST} PC plot of modern and prehistoric Asian groups 119

Figure 5-1: Histogram for Lokomotiv ^{14}C dates 157

Figure 5-2: ^{14}C dates for Sector 2 burials 158

Figure 5-3: Chronological distribution of Sector 2 burials 160

Table 5-5: MtDNA haplogroup distribution by cemetery sector at Lokomotiv .161

Table 5-6: mtDNA distribution between individuals in mass and single graves162

Table 5-7: mtDNA haplogroup distribution between non-communal graves at Lokomotiv 163

Table 5-8: mtDNA HVI lineages for non-communal graves at Lokomotiv 164

Figure 5-4: ^{14}C dates for human skeletal material from Ust-Ida....................169

Figure 5-5: ^{14}C chronology for burials at Ust-Ida 169

Figure 5-6: ^{14}C dates for Ust-Ida burials classified by typology....................170

Table 5-10: mtDNA haplogroup distribution for Serovo and Glazkovo individuals 172

Table 5-11: Serovo mtDNA haplogroup distributions by Ust-Ida cemetery cluster174

Table 5-12: mtDNA haplogroup distribution for Glazkovo burials by cemetery cluster....................174

Figure 5-7: ^{14}C dates for triple and communal interments at Ust-Ida 180

Table 5-14: DNA, ^{14}C and collagen yield data for Ust-Ida subadults....................181

List of Abbreviations

aDNA	ancient DNA
BP	years before present
bp	base pair
BSA	bovine serum albumin
DNA	deoxyribonucleic acid
mtDNA	mitochondrial DNA
nDNA	nuclear DNA
PCR	polymerase chain reaction
PMI	post mortem interval
RFLP	restriction fragment length polymorphism
HVI	hypervariable region I
PC	principal component
PCA	principal component analysis
R-matrix	relationship matrix
SOM	soil organic matter
STR	short tandem repeat
UV	ultraviolet

Chapter 1: Introduction

People leave a legacy of their lives behind when they die, chronicled both in their skeletons and in the grave where they are laid to rest. In burial environments lacking grave markers to give identity to those who are deposited beneath, human skeletal remains have the potential to reveal the population affinity, sex, age, stature, health, cause of death, status and occupation of an individual. Such information might be used to document the existence of a culture long vanished prior to the recording of history or to indict those committing crimes against humanity. By cataloguing the biological (*i.e.*, genetic) structure of a cemetery population, one can gain insight into the population origins of individuals interred within. Likewise, biological relationships between prehistoric and modern populations inhabiting the same region can potentially be explored. Furthermore, it may also be possible to examine whether kinship impacted social behaviour in a prehistoric group by seeking associations between biological and archaeological data.

This study explores how DNA markers retrieved from human skeletal material can help reconstruct the biological identity and social behaviour of mortuary populations. This research has been undertaken within the realm of the Baikal Archaeology Project (BAP), a large scale interdisciplinary initiative attempting to characterise the lives of prehistoric hunter-gatherer cultures who inhabited the Cis-Baikal region in the southwest of Eastern Siberia. The cold continental climate of Cis-Baikal was fundamental in preserving a multitude of prehistoric cemeteries across the region. This has provided BAP scholars with an exceptional opportunity to reconstruct the diets, demographic profiles and mortuary rituals of several different prehistoric Cis-Baikal cultures by examining their skeletal remains and associated archaeological assemblages.

A principal objective of BAP has been to explore the meaning of an observed hiatus in the archaeological record of the Cis-Baikal region. This gap was illuminated when radiocarbon dating demonstrated that roughly 800 years separated the existence of two Cis-Baikal groups, known as the Kitoi and Serovo-Glazkovo (**Table 1-1**). Scholars agree that the Kitoi and Serovo-Glazkovo, with different mortuary practices and subsistence strategies, represent distinct cultures (Weber, 1995; Katzenberg and Weber, 1999; Weber *et al.*, 2002). No overt signature of Kitoi culture has been detected in the post-hiatus Serovo-Glazkovo; thus it is believed that the hiatus began when the Kitoi disappeared from the region and ended with the emergence of the Serovo-Glazkovo culture. Consequently, the archaeological evidence suggests that the Kitoi and Serovo-Glazkovo are more likely to represent two discrete groups with divergent population origins rather than a common population who changed their cultural practices over time.

Table 1-1: Culture history model for prehistoric Cis-Baikal hunter-gatherer cultures (adapted from Weber et al., 2002)

Period	Culture/Mortuary Complex	Radiocarbon age BP
Late Mesolithic	Early Kitoi	8000–7000
Early Neolithic	Late Kitoi	7000–6100
Middle Neolithic	Hiatus	6100–5300
Late Neolithic	Early Serovo-Glazkovo	5300–4400
Bronze Age	Late Serovo-Glazkovo	4400–3300

This study explores if DNA polymorphic data, retrieved from Kitoi and Serovo-Glazkovo skeletal remains, can be used to reliably test whether these two cultures represent discrete populations. By characterising the genetic structures of the Kitoi and Serovo-Glazkovo, statistical algorithms may be applied to assess whether these two groups are different. It may also be possible to determine the population origins of each of these groups by comparing their DNA datasets with those of modern Siberians. Similar approaches have been used to explore relationships between prehistoric and modern North American populations (Parr *et al.*, 1996; Carlyle *et al.*, 2000; O'Rourke *et al.*, 2000; Stone and Stoneking, 1998; Kaestle and Smith, 2001); however, this study is the first to attempt the same with prehistoric and modern Siberian populations on a comparable scale. Furthermore, this research represents the oldest cemetery populations analysed for ancient DNA (aDNA) polymorphisms to date.

A secondary objective of this dissertation considers whether aDNA data can help to explain variation in burial patterning by looking for associations between mortuary ritual and biological data. Such an approach is largely unprecedented in aDNA research; however, analogous studies have been undertaken by physical anthropologists using phenotypic skeletal traits to serve as markers of biological relationships (e.g., Johnson and Lovell, 1994; Prowse and Lovell, 1995; 1996; Howell and Kintigh, 1996; Hemphill, 1999). These types of investigations are often referred to as biological affinities studies and have been used to examine how biological relationships between people in prehistoric communities influenced mortuary treatment, marriage practices and status acquisition.

The preservation of the Kitoi and Serovo-Glazkovo skeletal material analysed for this dissertation is exceptional given that the postmortem interval (PMI) for these specimens is as old as 8000 years. Regardless, it has been necessary to explore and design analytical strategies to optimise the retrieval and interpretation of aDNA from human remains to ensure that the DNA data retrieved from these two skeletal populations are authentic. The Kitoi and Serovo-Glazkovo skeletal remains were stored in a university anthropology collection subsequent to archaeological excavation and subject to osteological examination by a number of scholars. Thus, it has been essential to also understand how aDNA data are potentially compromised by contamination introduced through the handling of skeletal material and develop criteria to discriminate between exogenous contaminating DNA and the DNA endogenous to a sample.

While this dissertation focuses solely on the recovery and analysis of aDNA from archaeological populations, both methodological and theoretical aspects of this study have direct relevance to emerging topics in the medical sciences. Human tissue samples are frequently archived in medical laboratories and at times, archived samples are re-examined for molecular markers. As DNA in these archived samples is expected to degrade over time, identifying methods enhancing the recovery of degraded DNA will help ensure that the DNA retrieved is of sufficient quality and quantity to report results with a high degree of accuracy. By characterising factors influencing the decomposition of nucleic acids, laboratories may be able to better predict whether a sample contains sufficient DNA to justify analysis or to explain why DNA could not be successfully retrieved. It may also be possible to design storage methods to enhance the preservation of archived DNA.

Aspects of this study also have a bearing on issues in the forensic sciences, an area rooted in the discipline of Laboratory Medicine and Pathology. This research highlights the potential to identify efficient, cost-effective methods to retrieve and analyse DNA from human remains for the purpose of forensic investigation. Medical examiners are frequently called upon to identify degraded human remains from contexts such as mass graves or mass disasters. This includes identifying those killed in horrific genocide events that have been occurring throughout the world with alarming frequency. DNA identification has traditionally been used as a last resort in forensic cases as its current application is perceived to be both laborious and expensive. However, in circumstances where identification of degraded human remains cannot be made using dental records or x-rays, DNA may be the only means to obtain a positive identification. Identifying those killed in mass disaster and mass grave contexts not only provides resolution for families left behind but also imparts the names of the dead which are necessary to indict those responsible for their deaths.

This dissertation considers the analysis of ancient mitochondrial DNA (mtDNA) polymorphisms from the largest mortuary sample of this antiquity ever to be characterised. The body of this dissertation is divided into four chapters. Chapter 2 serves to frame the study of aDNA by discussing the theoretical and methodological aspects of this emerging discipline. This will include characterising the types of DNA markers typically used in aDNA studies and how normal postmortem modification of DNA impacts the retrieval of these markers from human skeletal material. As this dissertation considers the examination of prehistoric East Asian communities, this chapter also characterises the DNA markers defining modern East Asian populations. Typical methodological strategies to optimise aDNA retrieval will be explored as will criteria to determine the authenticity of aDNA data. Statistical algorithms that help assign meaning or significance to observed DNA distributions between or within cemetery populations will also be explored. This chapter will close by reviewing relevant aDNA studies that have been undertaken within both forensic and archaeological contexts.

Chapter 3 contains a study which discusses whether handling of skeletal material prior to aDNA analysis compromises the retrieval of endogenous mtDNA data or produces a DNA signature that could be misinterpreted as authentic. By assessing whether recognisable patterns emerge when skeletal material contaminated with modern DNA, this study proposes criteria to effectively discriminate between contaminant and authentic aDNA sequence data.

Chapter 4 describes the matrilineal character of the Kitoi and Serovo-Glazkovo and assesses the question of population replacement in the Cis-Baikal region during the Neolithic. Supplemental to the question of population replacement is whether modern Cis-Baikal populations are descended from either of these groups who inhabited the same region thousands of millennia prior. Chapter 5 takes a biological affinities approach to explore whether biological relationships influenced the spatial organisation of the cemetery populations examined in this study or the type of mortuary ritual given to an individual. This dissertation culminates with chapter 6 which summarises the major findings of this research and discusses directions for future work in the field of aDNA analysis.

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Chapter 2: Ancient DNA Analysis of Human Cemetery Populations

The biology of DNA

Every nucleated cell of the body contains two genomes; nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). While the cell nucleus holds two copies of nDNA, the copy number of mtDNA approaches hundreds to thousands per cell (Giles *et al.*, 1980; Wallace, 1995). Both nDNA and mtDNA markers have been used to study human remains. However, mtDNA polymorphisms have been examined more frequently in aDNA studies as its high copy number allows it to persist in postmortem tissue for longer than nDNA. This study predominantly considers the analysis of mtDNA polymorphisms; thus, this chapter will first address the biological aspects of mtDNA that dictate its use as a marker in human identity and population affinities studies. Later, nDNA markers of value for identifying sex and familial relationships will also be explored.

mtDNA

The mtDNA genome is a circular molecule containing 16 569 base pairs (bp) and codes for genes involved in mitochondrial function (Anderson *et al.*, 1981). The mtDNA genome was fully sequenced by Anderson and colleagues in 1981 and revised slightly by Andrews and others in 1999. The mtDNA genome consists of a highly conserved coding region, and a more polymorphic, non-coding region often referred to as the D-loop. Within the D-loop two distinct hypervariable (HV) regions, HVI and HVII are found; polymorphisms within these two regions form the basis for most population comparisons and will be discussed extensively within the context of Asian mtDNA variation.

The transmission of mtDNA from generation to generation differs from that of nDNA as mtDNA is thought to be passed exclusively through the maternal lineage (Giles *et al.*, 1980). Paternal mitochondrial contribution is believed to be circumvented at conception or during early embryogenesis, even if sperm mitochondria breach the ovum (*e.g.*, Kaneda *et al.*, 1995; Shitara *et al.*, 2000). However, mechanisms to eliminate sperm mtDNA are not failsafe as documented recently by Schwartz and Vissing (2002) who discovered both paternal and maternal mtDNA types in muscle tissue isolated from a man afflicted with mitochondrial myopathy. Interestingly, mtDNA isolated from the blood and fibroblasts of the proband contained only the maternal mtDNA type.

Whether mtDNA undergoes recombination is a contentious topic which has been hotly debated in the scientific literature over the last several years (e.g., Hagelberg *et al.*, 1999; Eyre-Walker *et al.*, 1999; Macaulay *et al.*, 1999; Bandelt *et al.*, 2001; Elson *et al.*, 2001; Hagelberg, 2003). Paternal mtDNA transmission (Schwartz and Vissing, 2002) could provide the conditions for recombination to occur as could the presence of nuclear pseudogenes, known to share homology with regions of mtDNA (Kamimura *et al.*, 1989). However, the likelihood of either of these events occurring with any sort of regularity is thought to be infinitely small (Elson *et al.*, 2001). Those supporting mtDNA recombination cite the occurrence of parallel mtDNA substitutions (*i.e.*, homoplasmy) in independent mtDNA lineages as evidence (e.g., Hagelberg *et al.*, 1999; Eyre-Walker *et al.*, 1999). Subsequently, Hagelberg and others' (1999) argument was compromised by errors in their mtDNA HVI dataset. Those who refute the likelihood of mtDNA recombination argue that homoplasmic sites in mtDNA are really hypervariable and represent positions that have increased mutation rates, regardless of the lineages in which they are found (e.g., Macaulay *et al.*, 1999; Stoneking, 2000; Elson *et al.*, 2001). As mtDNA recombination would have a tremendous impact on the decades of work that have been collected regarding the evolution and migration of modern Homo sapiens, this is an issue that clearly requires further study.

If one supports the paradigm that mtDNA is transmitted from generation to generation in a clonal fashion, mtDNA has great value as a marker to map the population history of females. The rapid mutation rate of mtDNA (ten to twenty times higher than nDNA; Wallace *et al.*, 1987) has allowed population-specific mutations to accumulate along maternal mtDNA lineages as females have diverged and migrated throughout the world (Wallace, 1995; Wallace *et al.*, 1999). Thus, the number of mtDNA substitution differences between any two individuals reflects the amount of time since a common maternal ancestor was shared.

Population studies of mtDNA variation were first undertaken more than two decades ago when Denaro and colleagues (1981) used restriction fragment length polymorphisms (RFLPs) to identify continent-specific RFLP variants in the coding region of mtDNAs from European, African and Asian populations. Higher-resolution RFLP studies of both coding and non-coding regions followed and identified African populations as having the most mtDNA diversity, suggesting that all humans evolved from a common maternal ancestor who came from Africa (Cann *et al.*, 1987). Since then, continental RFLP variation has been extensively characterised and the RFLP motifs defining unique mtDNA lineages have been organised into haplogroups (*e.g.*, Schurr *et al.*, 1992; Ballinger *et al.*, 1992; Torroni *et al.*, 1993; Torroni *et al.*, 1994). With the characterisation of the mtDNA coding region complete, researchers focused their efforts on describing the global variation of the 1021 bp D-loop through direct sequencing (*e.g.*, Shields *et al.*, 1993; Torroni *et al.*, 1994; Kolman *et al.*, 1996; Rando *et al.*, 1998; Macaulay *et al.*, 1999). As this study has focused specifically on the identification of Asian mtDNA markers, only the evolution of Asian mtDNA lineages will be discussed in further detail.

Asian mtDNA variation

All Asian mtDNA haplogroups fall into two large clusters often referred to as macro- or super-haplogroups (Kivisild *et al.*, 2002). One cluster, known as M (Chen *et al.*, 1995) is defined by a combined RFLP site gain at *DdeI* np 10394/*AluI* np 10397, first described by Ballinger and colleagues (1992). Within M, further RFLP changes create haplogroups C, D, G, G2a (formerly referred to as E) and Z (Kivisild *et al.*, 2002). Haplogroup C is defined by a *HincII* np 13259 site loss and *AluI* 13262 site gain (Torroni *et al.*, 1992); D by an *AluI* np 5176 site loss; G by a combined site gain at *HaeIII* np 4830/*HhaI* np 4831 (Torroni *et al.*, 1994), G2a by both the HincII/HhaI 4830/4831 gain in addition to a *CfoI* 7598 site loss (Ballinger *et al.*, 1992); and Z with a *HaeIII* site gain at np 16517 and *DdeI* np 11074 site gain (Schurr *et al.*, 1999).

The second clade, recently called N (Alves-Silva *et al.*, 2000), is broadly characterised by the loss of the *DdeI* np 10494/*AluI* np 10397 sites and includes haplogroups A, B, F and X (Brown *et al.*, 1998; Kivisild *et al.*, 2002;). Haplogroup A is characterised by a *HaeIII* site gain at np 663 (Torroni *et al.*, 1992; Ballinger *et al.*, 1992), B by a nine-bp deletion in the COII-tRNA^{Lys} gene (Cann and Wilson, 1983) and *HaeIII* np 16517 site gain; F by a *HincII* site loss at np 12406 (Ballinger *et al.*, 1992; Torroni *et al.*, 1993) and X by a *DdeI* 1715 site loss at np 1715. Haplogroup Y is also considered to be part of the N clade but is characterised by a site gain at *DdeI* np 10394, *MboI* np 7933 and *HaeIII* np 16517, as well as a site loss at *HaeIII* np 8391 (Stariskovskya *et al.*, 1998; Schurr *et al.*, 1999).

Further resolution of Asian mtDNA variation has been achieved by integrating RFLP motifs with sequence data from HVI (*e.g.*, Stariskovskya *et al.*, 1998; Schurr *et al.*, 1999; Derenko *et al.*, 2002; 2003; Kivisild *et al.*, 2002; Yao *et al.*, 2003) and to a lesser extent, HVII; (*e.g.*, Kivisild *et al.*, 2002; Yao *et al.*, 2003); allowing geographic-specific mtDNA haplogroup variants to be characterised. Table 2-1 outlines the major RFLP and HVI substitutions defining Asian mtDNA haplogroups. Polymorphisms characterised for HVII will not be presented as these were not examined in this study.

In addition to the *HaeIII* +663 site gain, founding haplogroup A lineages are defined by C-T transitions at np 16223, 16290 and a G-A transition at 16319 (Torroni *et al.*, 1993a). Haplogroup B has at T-C transitions at 16189, and 16217. The 16189 transition creates a homopolymeric tract of cytosines that can prove challenging to sequence (T. Schurr, *personal communication*). Haplogroup C is defined by C-T transitions at 16223 and 16327 as well as a T-C transition at 16298. Characteristic haplogroup D HVI substitutions include C-T transition at 16223 and a T-C transition at 16362; while haplogroup G2a is defined by C-T transitions at 16223 and 16278, a T-C transition at 16362 and an A-G transition at 16227. All haplogroup F lineages have a characteristic T-C transition at 16304 while haplogroup G lineages have transitions at 16017 (T-C), 16129 (G-A) and 16223 (T-C). Haplogroup Y has T-C transitions at 16189, 16231, and 16519 and a C-T transition at 16266; characteristic haplogroup Z transitions include A-G at 16129, C-T at 16185, 16223 and 16260 as well as T-C transitions at 16224, 16298 and 16519.

Table 2-1: Founding Asian mtDNA variants: All mtDNA lineages within each haplogroup will have these substitutions; additional substitutions will be built on this background and vary from lineage to lineage. Unless followed by a letter, all HVI substitutions represent transitions from the Cambridge Reference Sequence (CRS; Anderson et al., 1981).

Haplogroups		RFLPs	Asian Founder HVI Substitutions
M		DdeI +10394/AluI +10397	16223
	C	HincII -13259/ AluI +13262	16223 16298 16327
	D	AluI -5176	16223 16362
	G	HaeIII +4830/ HhaI +4831	16017 16129 16223
	G2a (E)	HaeIII +4830/ HhaI +4831, CfoI -7598	16223 16227 16278 16362
	Z	DdeI +11074, HaeIII +16517	16129 16185 16223 16224 16260 16298 165
N		DdeI -10394/AluI -10397	16223
	A	HaeIII +663	16223 16290 16319
	B	9-bp del np 8271-8281, HaeIII +16517	16189 16217
	X	DdeI -1715	16189 16223 16278
	F	HincII -12406	16304
	Y	DdeI +10394, MboI +7933, HaeIII -8391, HaeIII +16517	16189 16231 16266 16519

The geographic distribution of Asian mtDNA haplogroups provides clues about the population history of Asia and has been the subject of much inquiry over the last decade (*e.g.*, Ballinger *et al.*, 1992; Shields *et al.*, 1993; Torroni *et al.*, 1993b; Torroni *et al.*, 1994; Kolman *et al.*, 1996; Starikovskya *et al.*, 1998; Schurr *et al.*, 1999; Kivisild *et al.*, 2002). Asian mtDNA haplogroup distribution varies widely from south to north, supporting previous biological affinities studies that have identified a bifurcation between north and south Asian populations based on dental characteristics (*e.g.*, Turner *et al.*, 1987). South Asian populations have high frequencies of haplogroups B and F but generally lack the north Asian haplogroups A, C, D, G, X, Y and Z (Wallace *et al.*, 1999; Kivisild *et al.*, 2002) which largely define Siberian mtDNA distribution (Starikovskya *et al.*, 1998; Schurr *et al.*, 1999; Derenko *et al*, 2000; 2001; 2002; 2003). Asian populations at intermediate latitudes (*i.e.*, East and Central Asian groups, including those living in the steppe regions of Siberia) have the most heterogenous mtDNA distributions as they possess the full suite of Asian mtDNA haplogroups, perhaps earmarking this part of Asia as the region from which both Asian and North American populations were founded (*e.g.*, Kolman *et al.*, 1996).

The geographic distribution of mtDNA haplogroups within Asia and North America has also generated many questions considering the peopling of the Americas (*e.g.*, Schurr *et al.*, 1990; Torroni *et al.*, 1992, 1993a, 1993b; Shields *et al.*, 1993; Kolman *et al.*, 1996). Many studies have sought to illuminate the geographic origins of founder North American groups by identifying the modern Asian populations possessing haplogroups A, B, C, D and X, all of which are observed to be shared between Asian and North American populations, (Kolman *et al.*, 1996; Merriwether *et al.*, 1996; Stone and Stoneking, 1998; Derenko *et al.*, 2001). Although haplogroups A, B, C and D are predicted to have originated in Asia, it is not certain whether haplogroup X arrived in the Americas with Asian founder groups as it is also observed at small frequencies in Europeans (Brown *et al.*, 1998). However, the identification of haplogroup X in two discrete North American prehistoric samples has refuted the hypothesis that haplogroup X is present in North American groups as a consequence of recent European admixture (Malhi and Smith, 2002).

In the absence of any overt biological correlation between modern Asian, North and South American groups, the answers to the peopling of the Americas may rest in the mtDNA distributions of prehistoric skeletal populations from each locale. The characterisation of mtDNA haplogroup distributions for the Kitoi and Serovo-Glazkovo in this study highlights the feasibility of examining the population structure of prehistoric groups. However, it is unlikely that a comparative prehistoric North American population would ever be available to conduct such an analysis given the ethical considerations associated with the

North American Grave Repatriation Act (NAGPRA). Thus, the identity and origins of the founders of North America may never be known.

Nuclear markers for aDNA studies

Although nuclear DNA markers are more difficult to reliably recover from human skeletal material than mtDNA, they have been analysed in both forensic (Gill *et al.*, 1994; Corach *et al.*, 1997) and archaeological studies (Faerman *et al.*, 1998; Burger *et al.*, 1999; Matheson and Loy, 1999; Mays and Faerman, 2001; Keyser-Tracqui *et al.*, 2003). The amelogenin locus, found on both the X and Y chromosomes, has been frequently targeted in aDNA studies as a means of identifying the sex of human skeletal material (Gill *et al.*, 1994; Faerman *et al.*, 1998; Matheson and Loy, 1999; Gotherstrom, 1997; Mays and Faerman, 2001). Males and females are discriminated due to a 6-bp deletion found in the X-chromosome copy. When PCR is undertaken using amelogenin primers such as those designed by Mannucci and colleagues (1993), males will generate both a 106 and a 112 bp copy while females will produce two 106 bp copies. Amelogenin sexing was used to test hypotheses regarding the practice of differential infanticide at ancient Roman archaeological sites in both Israel and England (Faerman *et al.*, 1998; Mays and Faerman; 2001). A series of infant remains found below an ancient Roman bathhouse in Ashkelon, Israel were all determined to be male; thus leading Faerman and others (1998) to propose that prostitutes were selectively killing their newborn male children.

In 1998, Santos and others reported that the conventional amelogenin approach was potentially compromised by the presence of a rare deletion in the Y-copy of amelogenin which they had identified in 0.6 % of Sri-Lankan males. Males with this deletion would be falsely identified as female using the amelogenin assay; thus they proposed the use of a multiplex PCR approach using primers targeting both the amelogenin locus as well as another Y-chromosome-specific locus known as SRY. Some scholars have endorsed the use of SRY for forensic sex identification (*e.g.*, Santos *et al.*, 1998; Steinlechner *et al.*, 2002) but SRY has not yet been used to identify the sex of prehistoric human remains. DNA sexing was undertaken as part of this dissertation to determine the sex of the subadults from Lokomotiv and Ust-Ida, thus rounding out the demographic profile of these two cemetery populations. The results of the DNA sexing will be discussed in chapter 4. Confirmatory sexing of males was undertaken in a small subset of samples using the SRY locus (Santos *et al.*, 1998) to test the efficacy of this approach.

In addition to molecular sexing, a small number of forensic and archaeological studies have assessed the feasibility of analysing nuclear short tandem repeat (STR) markers which can potentially be used to identify biological relationships between individuals (*e.g.*, Gill *et al.*, 1994; Corach *et al.*, 1997; Burger *et al.*, 1999; Keyser-Tracqui *et al.*, 2003). The oldest skeletal material for which STR data have been reported date to the 1st millennium B.C. (Burger *et al.*, 1999; Keyser-Tracqui *et al.*, 2003). Keyser-Tracqui and colleagues (2003) used STR data to identify parent-offspring pairs in a Mongolian cemetery population dating to the 3rd century B.C. The postmortem decomposition of DNA, which will be discussed in detail below, compromises the successful retrieval of STR data from human remains. Allelic dropout is known to occur whereby a smaller allele will preferentially amplify over a larger one, which can result in a heterozygous individual appearing to be homozygous at that particular locus (*e.g.*, Burger *et al.*, 1999; Kaestle and Horsburgh, 2002).

While autosomal STR markers can be used to determine biological relationships between first-degree relatives, they will not identify those who are more distantly related. However, STRs are also found on the Y-chromosome and like mtDNA, may potentially be used to map the evolution of males throughout the world (*e.g.*, Seielstad *et al.*, 1998; Seielstad, 2000). By undertaking parallel mtDNA and Y-chromosome analysis in prehistoric cemetery populations, the population history of a group can potentially be reconstructed. Keyser-Tracqui and colleagues (2003) identified a series of matrilineages and patrilineages in their Mongolian cemetery population, including one representing a group of males buried in a discrete sector of the cemetery. Comparing mtDNA and Y-chromosome diversity in a group also has the potential to reveal post-marital residence patterns for prehistoric populations (*e.g.*, Williams *et al.*, 2002). If females are observed to have more mtDNA diversity than the Y-chromosome diversity observed for males, it is likely that patrilocal post-marital residence was practiced by the group.

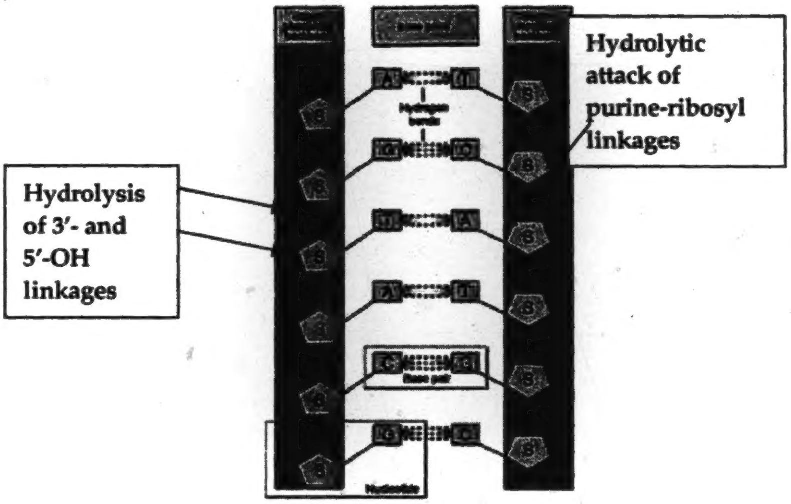
The chemistry of ancient DNA

During life, the integrity of DNA is maintained through intracellular repair mechanisms (Lindahl, 1993; 1996). With death, DNA repair ceases and irreversible DNA damage begins. The mechanisms by which nucleic acids are modified in postmortem tissue have been well characterised (*e.g.*, Rogan and Salvo, 1990; Lindahl, 1993; 1996) and fall into the general categories of hydrolysis and oxidation.

Hydrolysis

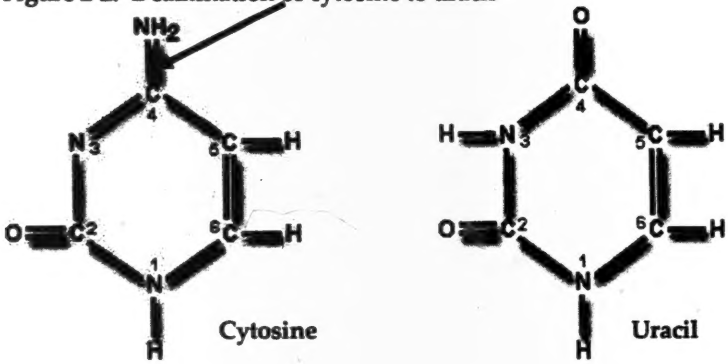
The postmortem hydrolysis of DNA occurs through enzyme activity or with the production of acid metabolites. Tissue autolysis occurs immediately following death with the release of endogenous hydrolases from lysosomes (Bar *et al.*, 1988). Putrefaction by microorganisms destroys DNA through enzyme activity and the production of organic acid byproducts (Bar *et al.*, 1988; Vass *et al.*, 1992). Both autolysis and putrefaction cause fragmentation of DNA. Depurination (*i.e.*, loss of guanine or adenine) of DNA is the most common mechanism of DNA decay (Lindahl, 1993), and occurs when glycosyl bonds joining the ribose and purine groups are attacked. Apurinic sites promote fragmentation of DNA through hydrolytic destruction of the 3′- or 5′-phosphodiester linkages (Eglinton and Logan, 1991; Lindahl, 1993). The principal sites of hydrolytic attack on DNA are illustrated in Figure 2-1.

Figure 2-1: Principal sites of hydrolytic attack on DNA


Hydrolytic attack of purine-ribosyl linkages
Hydrolysis of 3′- and 5′-OH linkages
A
T
Hydrogen bonds
G
C
T
A
A
T
C
G
Base pair
G
C
S
Nucleotide

Hydrolysis of DNA can also create miscoding lesions in DNA template molecules. The hydrolytic deamination of cytosine, shown in Figure 2-2 creates uracil which will cause a C-G base pair to be coded as an A-T base pair during the subsequent DNA replication of this template (Lindahl, 1993; Hoss *et al.*, 1996; Hofreiter *et al.*, 2001)

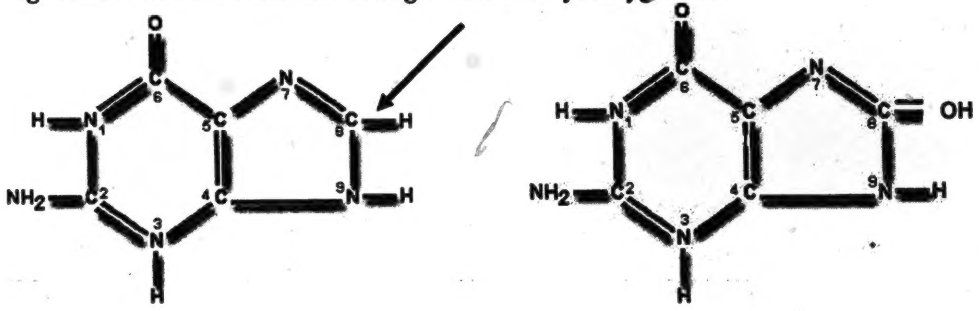
Figure 2-2: Deamination of cytosine to uracil



Oxidation

Nucleic acids are readily oxidized by free radicals produced from exposure to ionizing radiation (Hutchinson, 1985; Eglinton and Logan, 1991, Lindahl, 1985; 1993). Pyrimidines are the preferential substrate for oxidative attack but purines are also targeted. In vitro studies have shown thymine adducts to form in DNA exposed to ultraviolet (UV) irradiation (Ou *et al.*, 1991); oxidative damage to thymine also creates the pyrimidine derivative 5-hydroxy-5-methyl hydantoin (*e.g.*, Hoss *et al.*, 1996; Fattorini *et al.*, 2000). Unlike oxidative pyrimidine modification, purines are generally oxidized through hydroxyl attack (Lindahl, 1993; Hoss *et al.*, 1996). The major purine product created by oxidative modification is 8-hydroxyguanine. The oxidation of guanine to 8-hydroxyguanine shown in Figure 2-3 can potentially impact the interpretation of retrieved DNA data and will be discussed further in the context of aDNA analysis.

Figure 2-3: Oxidative conversion of guanine to 8-hydroxyguanine



Guanine

8-OH- Guanine

Characterising the postmortem persistence of DNA

The preservation of DNA in post mortem tissue is contingent on two factors: the physiochemical properties of tissue and the degree of environmental assault. The following section discusses the significance of variables thought to influence DNA integrity in the early and extended postmortem interval (PMI). In doing so, the differential decay of DNA in differing depositional environments may be better explained.

Physiochemical properties of DNA in tissue

The post mortem persistence of DNA varies greatly with tissue type. Although DNA has been isolated from mummified soft tissue in exceptional circumstances (*e.g.*, Paabo *et al.*, 1988; Handt *et al.*, 1996), most soft tissue DNA is rapidly degraded in early PMI (Bar *et al.*, 1988; Hochmeister *et al.*, 1991). Therefore, DNA analysis of human remains is most often attempted from hard tissue (*e.g.*, Hagelberg *et al.*, 1989; 1991; Holland *et al.*, 1991; Colson *et al.*, 1997; Stone and Stoneking, 1998) and thus, will be the principal focus of this study.

DNA will persist in bone and teeth over hundreds to thousands of years (*e.g.*, Stone and Stoneking, 1998; Burger *et al.*, 1999; Meyer *et al.*, 2000; Carlyle *et* *al.*, 2000; Kaestle and Smith, 2001; Keyser-Tracqui *et al.*, 2003). DNA in hard tissue is bound to hydroxyapatite, ([Ca5 (PO4)3 OH]; Hochmeister *et al.*, 1991) and studies demonstrate hydroxyapatite to conserve the integrity of DNA in post mortem tissue (Lindahl, 1993; Gotherstrom, 2002). Robins and Lindahl (unpublished, cited in Lindahl, 1993) observed the rate of DNA depurination to decrease by 50 % in DNA bound to hydroxyapatite compared to unbound DNA. Hydroxyapatite concentrations vary with tissue type (Newesley, 1989). For example, tooth enamel is composed of 95 % hydroxyapatite while bone contains 70 % (Wainwright, 1976). Thus, DNA in teeth may be better protected from post mortem modification. However, teeth are less frequently used than bone in aDNA research as they are highly valued for use in isotope and biological affinities studies by physical anthropologists. Therefore, most aDNA studies have focussed on the retrieval of DNA from bone.

Both compact and cancellous (*i.e.*, spongy) bone have been used in aDNA studies although it remains unclear which yields the greatest quantity of DNA. Henderson (1987) reported that bone with a high ratio of compact to cancellous bone (*e.g.*, femur) might better protect DNA from postmortem change. However, Lee and others (1991) demonstrated that DNA yields from spongy bone were 10 to 20 times higher than the DNA yield from a comparative mass of compact bone. Therefore, the best bone samples for aDNA studies may be derived from spongy bone encased within an outer matrix of compact bone.

Differences in DNA preservation between adult and juvenile bone are expected as adult bone has a higher hydroxyapatite content than juvenile bone (Henderson, 1987). However, the higher cellularity of juvenile bone (Vaughan, 1981) may mitigate the decreased levels of hydroxyapatite, making the retrieval of DNA from juvenile bone to be as likely as that from adult bone (Colson *et al.*, 1997). Stojanowski and colleagues (2002) found no significant difference in overall bone preservation between juvenile, adult and senescent groups from a 7000 year-old mortuary site.

Surprisingly, there is little correlation between the PMI of the tissue and the quality of DNA retrieved. Lindahl (1993) estimates DNA exposed to moderate temperatures will degrade to small fragments in the order of 10^5 years. However, studies have retrieved informative DNA sequences from 7000-year old hard tissue (*e.g.*, Meyer *et al.*, 2000; Mooder *et al.*, 2003) whereas, similar attempts from much younger tissue have failed (*e.g.*, Cattaneo *et al.*, 1997). This differential DNA preservation illustrates the influence the depositional environment has on the long-term persistence of DNA in hard tissue; this will be discussed in detail in the following sections.

Environmental influences on aDNA preservation

Temperature

Every 10 °C increase in temperature is predicted to double a chemical reaction rate (Von Endt and Ortner, 1984). Thus, temperature has a significant effect on the post mortem preservation of DNA. Autolysis and putrefaction will accelerate with increased temperature (Bar *et al.*, 1988; Vass *et al.*, 1992); therefore DNA decay in early PMI is also predicted to increase. Similarly, microbiological activity in soil will accelerate at soil temperatures greater than 10 °C (Paul and Clark, 1989).

In contrast, the rate of DNA decay is predicted to decrease by 10 to 25 times for every 20 °C decrease in temperature (Hoss *et al.*, 1996). Hoss and others (1996) have shown DNA from tissues deposited in arctic and subarctic environments are subjected to minimal oxidative damage. Buried bodies are predicted to decay at a slower rate than bodies deposited on the surface (Bass and Rodriguez, 1985), and this effect is enhanced with increasing burial depth. Seasonal temperature fluctuations may also influence decay rates of human bodies (Henderson *et al.*, 1987), but studies are rare.

Water

Following death, DNA degradation begins with the hydrolysis of phosphodiester bonds by water intrinsic to bone and DNA structure (Von Endt and Ortner, 1984; Lindahl, 1993). Depositional environments with waterlogged soils (Henderson *et al.*, 1987) or increased humidity (Holland *et al.*, 1991; Goodwin *et al.*, 1999) will promote hydroxyapatite dissolution and thus, DNA degradation. The degradation rate of DNA in hard tissue exposed to water has not been thoroughly characterised; however, interplay with other environmental variables including pH and temperature are certain (Eglinton and Logan, 1990).

Depositional contexts where water is predicted to promote DNA preservation are rare. As microbiological activity is limited in waterlogged environments (Paul and Clark, 1989), short term DNA preservation may be promoted in hard tissues where immersion in water occurred shortly after death. DNA from mummified tissues is often heavily oxidized (Paabo, 1989). Lindahl (1993) speculates desiccated DNA is more susceptible to oxidative decay. The high salinity of seawater may also preserve the integrity of DNA. Lindahl (1993) predicts the rate of DNA decay to decrease by five- to ten-fold in an environment of high ionic strength over that in an isotonic environment. Likewise, Weedn (1998) reports DNA identification was successful in using bone fragments retrieved from the ocean weeks after the crash of TWA flight 800.

pH and soil type

When exposed to acid, hydroxyapatite in hard tissue will be modified to more highly soluble forms, causing the dissolution of the inorganic matrix (Piepenbrink, 1989). The loss of hydroxyapatite destroys bone microstructure, creating cracks, thus exposing the inner matrix of hard tissue to hydrolytic attack by acids. As such, DNA in bone and teeth is predicted to suffer a similar fate (Eglinton and Logan, 1990).

In early PMI, acids generated from autolytic and putrefactive processes are mainly responsible for the loss of DNA phosphodiester linkages. In extended PMI, acid hydrolysis of DNA will continue if exposed to sandy soils (Piepenbrink, 1989), rainwater (Henderson, 1987) or soil organic matter (SOM; Child, 1995). The preservation of DNA may be promoted in environments where the pH is neutral or slightly alkaline (Lindahl, 1993), such as are typically found in clay soils or limestone-derived sediments (Paul and Clark, 1989). As the native structure of hydroxyapatite is conserved in bone at a pH of 7 to 7.5 (Piepenbrink, 1989), DNA within hard tissue may be better protected from environmental assault in neutral or slightly alkaline environments.

Radiation

The effects of UV and ionizing radiation on oxidative DNA decay have been described through in vitro studies (*e.g.*, Hutchinson, 1985; Ou *et al.*, 1991; Lindahl, 1993). However, the relationship between post mortem DNA integrity and long term exposure to radiation has not been explored. UV radiation damages DNA primarily through the formation of thymine adducts (Ou *et al.*, 1991). As adduct formation prevents hydrogen bonding between complementary bases, enzymatic amplification of DNA may be compromised.

Ionizing radiation causes DNA damage through direct exposure to radioactivity or indirectly through free radical generation (Hutchinson, 1985). Human remains buried in clay rich soils may be exposed to ionizing radiation through the radioactive decay of minerals common to clay (Eglinton and Logan, 1990). DNA decay in waterlogged clay soils may be exacerbated as ionized water will generate free radicals (Hutchinson, 1985). Rogan and Salvo (1990) speculate DNA decay by ionizing radiation may be limited if abundant SOM is present. Antioxidants produced by microorganisms in SOM are predicted to trap free radicals preventing oxidative attack of DNA. Long term preservation of DNA in environments dominated by clay and SOM has not been described.

Microorganism activity

Both putrefactive microorganisms and soil microorganisms cause significant DNA decay in both soft and hard tissue (Child; 1995). Hydrolytic and oxidative modification of DNA occurs through enzymatic action (both endonucleases and exonucleases), organic acid production and the generation of free radicals (Rogan and Salvo, 1990). Although the inner matrices of bone and teeth should be protected against initial assault, putrefaction will target both hard and soft tissue (Child, 1995). Child (1995) predicts putrefaction causes variable decomposition of hard tissue. Bones surrounding the thoracic cavity (*e.g.*, vertebrae and ribs) may not withstand putrefactive assault to the same degree as bones outside of the thorax (*e.g.*, long bones and skull).

Both bacteria and fungi are ubiquitous in soil and microorganisms are commonly isolated from hard tissue associated with soil (Piepenbrink, 1989; Child 1995). Microorganism activity in soil is highest where SOM is abundant (Paul and Clark, 1989) and pH is neutral (Eglinton and Logan, 1990). In contrast, soil microbial activity is seen to decrease with increasing soil depth and decreasing soil temperature.

Microorganism activity is strongly associated with the appearance of adipocere in post mortem tissue. Adipocere consists of fatty acids modified from adipose tissue in damp environments (Pfeiffer *et al.*, 1998). The relationship between adipocere and DNA integrity is unknown. However, Graw and others (2000) were unable to amplify either nDNA or mtDNA from bone modified by adipocere. Likely, the acidic character of adipocere, together with the presence of microorganisms, exacerbates the decay of DNA.

Studies of taphonomic DNA decay

The taphonomic alteration (*i.e.*, postmortem modification) of human remains in various depositional contexts has been thoroughly examined (*e.g.*, Rodriguez and Bass, 1985; Vass *et al.*, 1992). However, these studies have focussed on gross tissue decomposition rather than DNA decay. Many archaeological studies have observed a correlation between the degradation of hydroxyapatite and collagen in bone (*e.g.*, Piepenbrink, 1989; Gotherstrom, 2002); however, the relationship to DNA decay is indirect. Of the studies directly assessing the degree of DNA decay in human bone, most are retrospective (*e.g.*, Holland *et al.*, 1993; Burger *et al.*, 1999). The variables influencing the observed degree of DNA decay are extrapolated from the environment in which the remains are discovered. Controlled experiments designed to assess DNA decay in different depositional environments have been described (*e.g.*, Pfeiffer *et al.*, 1998; Frank *et al.*, 1999). The experimental parameters used in these studies are both poorly defined and inadequate in scope. Studies of greater depth are necessary to predict the means by which DNA decay is accelerated in various depositional environments. By characterising DNA decay, analytical parameters may be better designed to optimise the retrieval of DNA from human remains.

The manipulation of aDNA

Postmortem modification of DNA creates many challenges for aDNA analysis. Although the polymerase chain reaction (PCR: Sakai *et al.*, 1985; Mullis, and Faloona, 1987) revolutionised the analysis of aDNA, its application is not foolproof; thus, much research dedicated to the use of PCR in aDNA analysis has been published in the last decade (*e.g.*, Paabo, 1989; Paabo *et al.*, 1990; Handt *et al.*, 1994; Handt *et al.*, 1996; Hofreiter *et al.*, 2001). Generating PCR product from postmortem DNA is only one hurdle to be surmounted as the successful retrieval of PCR product does not guarantee its authenticity (*e.g.*, Paabo *et al.*, 1990; Handt *et al.*, 1996; Fattorini *et al.*, 2000; Hoffreiter *et al*, 2001). Thus, this next section will discuss both specific strategies proposed to retrieve aDNA from postmortem skeletal material and those designed to guarantee the authentic, endogenous nature of the retrieved aDNA data.

Ancient DNA analytical strategies

Hagelberg and colleagues (1989) were among the first to report the successful retrieval of aDNA from archaeological bone. Since then, much research has been devoted to optimising the extraction of DNA from hard tissue (e.g., Hoss and Paabo, 1993; Evison *et al.*, 1997; Cattaneo *et al.*, 1997). The prevailing extraction method used in aDNA studies was first described by Boom and colleagues (1990) and modified for hard tissue by Hoss and Paabo (1993). This method, which utilises guanidium thiocyanate (GuSCN) as a chaotropic agent, is observed to be highly effective in eliminating PCR inhibitors (Hoss and Paabo, 1993; Cattaneo *et al.*, 1997; Evison *et al.*, 1997). The GuSCN method is thought to be superior to the conventional phenol-chloroform DNA extraction method (e.g., Sambrook *et al.*, 1989) as exposure to organic solvents is reduced and potential inhibitors are washed away rather than co-extracted (Kaestle and Horsburgh, 2002). Some studies also advocate that bone be pretreated with EDTA to decalcify tissue prior to extraction with GuSCN. Although the exact mechanism has not been described, EDTA treatment is thought to increase the yield of aDNA recovered from bone (Colson *et al.*, 1997; Evison *et al.*, 1997; Kaestle and Smith, 2000).

PCR amplification of aDNA is technically fastidious compared to modern DNA; as such, several modifications for aDNA PCR mixtures have been proposed. Bovine serum albumin (BSA) is a common component of aDNA PCR as it is thought to bind inhibitors in aDNA extracts (Hagelberg *et al.*, 1989; 1991; Holland *et al.*, 1991). Early aDNA studies used hot-start PCR which involved the use of a wax plug to separate reaction components from Taq polymerase and the template prior to the initial denaturation of the template (*e.g.*, Handt *et al.*, 1996; Krings *et al.*, 1997 Stone *et al.*, 1998). Hot-start PCR improved PCR specificity by preventing mispriming of *Taq* polymerase on the template before it was fully denatured. Hot-start methods have since been replaced with antibody-linked *Taq* polymerases (*e.g.*, Platinum *Taq*; Invitrogen) that also improve PCR specificity by delaying the activation of Taq polymerase. Conventional PCR troubleshooting techniques (*e.g.*, altering Mg^{2+}, nucleotide or *Taq* polymerase concentrations) have also been mentioned in several degraded DNA studies (*e.g.*, Evison *et al.*, 1997; Vernesi *et al.*, 1999) as a means of improving the sensitivity or specificity of the PCR reaction.

PCR primers for aDNA analysis should be designed to target template regions less than 200 bp in length to circumvent the effects of DNA fragmentation (*e.g.*, Paabo, 1989; Krings *et al.*, 1997). Semi-nested or nested PCR approaches are generally necessary to amplify ancient templates greater than 200 bp in length (*e.g.*, Jehaes *et al.*, 1998; 2001) and involve the reamplification of a PCR template with one (semi-nested) or two (nested) primers internal to the primary PCR primers to improve detection sensitivity (Diffenbach and Dveksler, 1995). Nested PCR techniques can potentially introduce contamination when the primary PCR vessel is opened to add the nested primers (*e.g.* Kolman and Tuross; 2000). It may be possible however, to design a multiplex nested PCR where primers with differing T_m are used; if the outer primer pair anneal at a higher temperature than the inner primer(s), both amplifications can proceed without reopening the reaction vessel (Diffenbach and Dveksler, 1995). An alternative approach to nested PCR uses multiple primers to create a series of overlapping fragments that are generated through several independent PCR. Many aDNA studies examining mtDNA sequence variation have used an overlapping fragment strategy to amplify the mtDNA HVI and HVII, which at approximately 400 bp each, are too large to amplify in a single PCR (*e.g.*, Handt *et al.*, 1996; Colson *et al.*, 1997).

Challenges to aDNA authenticity

Postmortem DNA damage has the capacity to inhibit PCR and can potentially generate non-authentic PCR products (Paabo, 1989; Paabo *et al.*, 1990; Handt *et al.*, 1994; Handt *et al.*, 1996; Kolman and Tuross, 2000; Cooper and Poinar; 2001; Hofreiter *et al.*, 2001). PCR inhibition occurs when DNA polymerases extending along template molecules encounter oxidized pyrimidine products (C or T) or sites where purines (G or A) have been lost through hydrolysis (Lindahl, 1993; Hoss *et al.* 1996; Fattorini *et al.*, 1999). The association between the presence of oxidized pyrimidines in a DNA template and PCR inhibition has been demonstrated in a study by Hoss and colleagues (1996) who found three- to thirty-fold fewer modified pyrimidines (mostly thymine derivatives) in tissues containing amplifiable DNA than in tissues where PCR amplification failed.

PCR inhibition can also result in a phenomenon known as jumping PCR where partially extended PCR fragments recombine to create mosaic PCR products (Paabo *et al.*, 1990; Handt *et al.*, 1994; Handt *et al.*, 1996). DNA fragmentation, occurring subsequent to hydrolytic depurination (*i.e.*, loss of purines), also compromises PCR by reducing the length of the PCR template available for amplification (Paabo, 1989; Lindahl., 1993). It may be possible, however, to reduce the effects of DNA fragmentation with appropriate experimental design. Several studies have reported an inverse relationship between template length and amplification success (Paabo, 1989; Handt *et al.*, 1994). By designing primers to target short regions of template, DNA may still be amplified from tissues that have suffered significant hydrolytic assault.

Miscoding lesions created through hydrolytic and oxidative attack on DNA bases have a great impact on the retrieval of authentic aDNA data (Lindahl, 1993; Hoss *et al.*, 1996); as previously mentioned, these occur with the modification of both cytosine and guanine in postmortem DNA (Lindahl, 1993; Hoss *et al.* 1996; Cooper and Poinar, 2000; Fattorini, 2000; Hofreiter, 2001; Gilbert *et al.*, 2002). Deamination of adenine to hypoxanthine causing a base-pair shift from A/T to G/C is also known to occur; however Lindahl (1993) estimates that the rate of adenine deamination is very small compared to that of cytosine deamination.

As postmortem DNA damage is observed to occur randomly from template to template (*e.g.*, Hofreiter *et al.*, 2001), the likelihood that hydrolytic and oxidative lesions will impact the retrieval of authentic aDNA data are predicted to be dependent on the number of endogenous DNA template molecules present in a PCR (Handt *et al.*, 1996; Cooper and Poinar, 2000; Hofreiter *et al.*, 2001). If the template number is small, mutagenic lesions may be incorporated into the template sequence in the early stages of PCR, creating a DNA sequence with non-authentic substitutions. Therefore, it is generally believed that aDNA PCR containing less than a hundred template molecules are likely to produce non-authentic DNA data (Handt *et al.*, 1996; Hofreiter *et al.*, 2001). Competitive PCR methods to quantitate the number of aDNA molecules available for amplification have been employed in several studies (*e.g.*, Handt *et al.*, 1994a; 1994b; 1996; Krings *et al.*, 1997). Competitive PCR involves the use of a synthetic template that differs in size from the ancient template and has a known copy number. When both the competitor and ancient template are incorporated in a PCR mix, the molecule present in the highest quantity will be preferentially amplified. Both templates will amplify equally if they are present in similar copy number; because the copy number of the competitor is known, the copy number of the aDNA template can be extrapolated. Specific competitive PCR methods will not be discussed in further detail as they were not undertaken as part of this study. Interested readers instead are invited to consult the comprehensive review by Reischl and Kochanowski (1995).

Contamination

Aside from postmortem DNA modification, the greatest challenge faced by the aDNA analyst is contamination of aDNA extracts with exogenous, modern DNA. As such, all aDNA studies must be designed to ensure that DNA retrieved from human remains is intrinsic to the sample. Using mtDNA markers in aDNA studies increases the likelihood of contamination due to its copy number being three to four orders of magnitude higher than that of nDNA (Wallace, 1987). Contamination may be introduced in the laboratory (*e.g.*, Handt *et al.*, 1994; Handt *et al.*, 1996; Kolman and Tuross, 2000) or prior to analysis during archaeological excavation or curation (*e.g.*, Brown and Brown, 1992; Kaestle and Horsburgh, 2002). If skeletal remains are poorly preserved, handling during excavation or curation may transfer sufficient mtDNA to outcompete the DNA endogenous to the bone during PCR (Kolman and Tuross, 2000; Mooder, Chapter 3). Therefore, it is important that skeletal samples destined for aDNA analysis are excavated wearing gloves (Brown and Brown, 1992; Colson *et al.*, 1997; Keyser-Tracqui *et al.*, 2003). If aDNA analysis is desired using curated skeletal remains, it is crucial that reference DNA samples be collected from all individuals known to have handled the bones so that contamination, if present can be identified.

Many strategies have been explored to circumvent contamination of aDNA samples in the laboratory. First, the areas in which pre- and post-PCR manipulations are undertaken must be physically separated to prevent the opportunity for PCR amplicons to contaminate aDNA manipulations (*e.g.*, Hoss and Paabo, 1993; Stone and Stoneking, 1998; Cooper and Poinar, 2000; Hofreiter *et al.*, 2001). Dedicated equipment and supplies must be used for aDNA manipulations while wearing appropriate protective wear (*i.e.*, gloves, face mask, hair net and gown) to reduce the opportunity for contamination. Chemical and physical methods are regularly employed in aDNA laboratories to remove contaminating DNA from equipment, supplies and samples. Such methods include UV irradiation (Ou *et al.*, 1991) bleach (Prince and Andrus, 1992) and sample surface removal (*e.g.* Hedges *et al.*, 1995; Stone and Stoneking, 1998; Kaestle and Horsburgh, 2002). UV irradiation is known to form dimers between adjacent thymine residues in DNA, thus inhibiting amplification (Breimer and Lindahl, 1985; Ou *et al.*, 1991). Therefore, UV irradiation is used to destroy exogenous DNA contamination in reagents used for aDNA analysis (*e.g.*, Handt *et al.*, 1994; Kolman and Tuross; 2000).

The exact mechanism by which bleach degrades DNA is not completely understood but is observed to induce both oxidative and hydrolytic damage to DNA templates (Prince and Andrus, 1992). Bleach is typically used in aDNA laboratories at varying concentrations to decontaminate work spaces (*e.g.*, laminar flow hoods and benches), laboratory supplies (*e.g.*, pipettors, racks, tip boxes etc.) and aDNA samples (*e.g.*, Handt *et al.*, 1994; Hedges *et al.*, 1995; Stone and Stoneking, 1998; Kaestle and Smith, 2001; Kolman *et al.*, 2000). Removing several millimetres of bone surface is also thought to eliminate contaminating DNA, and is generally accomplished using sharp or abrasive materials (*e.g.*, Hedges *et al.*, 1995; Stone and Stoneking, 1998; Keyser-Tracqui *et al.*, 2003). Autoclaving is also commonly employed in aDNA laboratories to remove contaminating DNA from laboratory reagents and equipment (*e.g.*, Keyser-Tracqui *et al.*, 2003; Mooder, unpublished). The use of DNase I to destroy contaminating DNA in PCR mixes has recently been proposed (Eshleman and Smith, 2001) and holds promise as DNase I treatment is the only decontamination protocol that is known to not degrade Taq polymerase.

No single aDNA decontamination strategy has been proven to be entirely effective (*e.g.*, Hedges *et al.*, 1995; Kolman and Tuross, 2000); therefore, ancient DNA studies must incorporate controls and mechanisms to identify contamination when it does occur. Negative extraction and PCR controls are instrumental in detecting systematic contamination introduced during aDNA manipulation (*e.g.*, Handt *et al.*, 1994; Jehaes *et al.*, 1998; Hofreiter *et al.*, 2001). However, such controls are less likely to detect contamination affecting individual samples (*i.e.*, random contamination); therefore, additional criteria including the reproduction of aDNA data from independent extracts and multiple amplifications are necessary (*e.g.*, Poinar and Cooper, 2000; Hofreiter *et al.*, 2001). Strategies to detect and characterise aDNA contamination will be discussed in further detail in Chapter 2.

Establishing the authenticity of aDNA

Cloning PCR products for mtDNA sequencing instead of directly sequencing mtDNA PCR products has been advocated by some scholars to be the most appropriate approach in retrieving mtDNA sequence data from postmortem tissue (e.g., Handt *et al.*, 1996; Cooper and Poinar, 2000; Hofreiter *et al.*, 2001). The foundation of this argument considers that directly sequencing PCR templates produces a sequence that reflects the PCR product present in the highest quantity. As a consequence, PCR products containing a high ratio of modified to undamaged template molecules can potentially yield unauthentic data if sequenced directly. DNA derived from individual clones represent single templates from PCR products. Miscoding lesions in mtDNA sequences retrieved from clones will be observed as non-reproducible substitutions which vary from clone to clone. Thus, an authentic mtDNA sequence can be derived from a series of clones by compiling a consensus sequence containing only the substitutions observed across all clones.

In addition to the cloning of PCR products, there are several criteria that can be applied to discriminate authentic and non-authentic aDNA polymorphism data from postmortem tissue (*e.g.*, Cooper and Poinar, 2000; Kolman and Tuross, 2000; Hofreiter *et al.*, 2001; Poinar, 2003). These criteria will be discussed in detail in Chapter 3.

Population genetics and the statistical interpretation of aDNA data

Overview

Ancient DNA data retrieved from human skeletal populations have little meaning in the absence of statistical estimations which allow relationships within and between populations to be described. There are many statistical algorithms designed to estimate intra- and inter-population affinities (*e.g.* Nei, 1987; Weir, 1990; Cavalli-Sforza *et al.*, 1995; Hedrick, 2000; Nei and Kumar, 2000; Felsenstein, 2002). The population genetic methods discussed in these volumes have been routinely applied in the analysis of contemporary populations and in certain cases, have been extrapolated to aDNA data sets.

The statistical analysis of ancient DNA population data is rather inexact and certain caveats are required regarding the validity of the interpretations achieved. The level of statistical rigour attained when only mtDNA variation is examined in an ancient population is lower than studies that examine multiple DNA markers as the mtDNA genome is considered to be a single locus (Nei, 1987; Nei and Kumar, 2000). While mortuary populations tend to be treated as discrete population samples, the temporal boundaries of cemeteries often span hundreds or thousands of years (*e.g.* O'Rourke *et al.*, 2000; Kaestle and Smith, 2001; Kaestle and Horsburgh; 2002). Thus, erroneous interpretations of prehistoric population structure are potentially made when analogies are sought in contemporary populations defined by only a few generations.

Seeking ancestral-descendant relationships between prehistoric and modern populations needs also to be approached cautiously as cemetery populations do not always represent all members of a group (*e.g.*, Larsen, 1997: p. 334; Link, 1996; Weber *et al.*, 2002). Children are often buried outside formal cemeteries and if cemeteries were only used sporadically from generation to generation, people may have been buried in more than one location. Such behaviour can potentially under represent the true degree of genetic diversity in a prehistoric population. However, if aDNA data are found to be relatively heterogenous and distributed evenly across the temporal boundaries of the cemetery's use, it may be possible to derive meaningful statistical associations from population comparisons (O'Rourke *et al.*, 2000; Kaestle and Smith, 2001; Kaestle and Horsburgh; 2002). The following section discusses how statistical algorithms can be potentially applied to the analysis of aDNA population data to answer questions about relationships between prehistoric and modern populations occupying the same geographic region and the intra-population structure of prehistoric groups.

Inter-population comparisons

Of all the potential statistical algorithms used for modern and prehistoric population studies, those estimating the biological (*i.e.*, genetic) distances between populations are among the most frequently applied (Cavalli-Sforza *et al.*, 1996). Biological distance estimates operate under the assumption that populations with similar genetic structures are more likely to have exchanged genes than those with disparate genetic distributions (Kaestle and Smith, 2001; Kaestle and Horsburgh, 2002). Biological distances between populations are predicted to increase or decrease as a function of geographic proximity and time since a common ancestor was shared (Cavalli-Sforza *et al.*, 1996; Relethford, 1996; Hedrick, 2000).

There are two different models underlying biological distance algorithms; those that assume that populations differentiate through mutation (*e.g.*, Sokal and Michener, 1958; Saitou and Nei, 1987), and others operating under mechanisms of gene flow and genetic drift (*e.g.*, Wright, 1951; Harpending and

Jenkins, 1973; Reynolds *et al.*, 1983; Weir, 1990). Biological distance comparisons using mutation models are used to estimate the evolutionary history of a set of populations. Characterising the degree of divergence occurring between populations through the mechanism of genetic mutation allows the time since two groups shared a common ancestor to be estimated (Nei, 1987; Hedrick, 2000; Nei and Kumar, 2000). Evolutionary relationships between populations based on mtDNA data are generally represented in the form of phylogenetic trees (*e.g.*, Nei, 1987; Cavalli-Sforza *et al.*, 1996; Nei and Kumar, 2000; Felsenstein, 2002) or networks (Bandelt *et al.*, 1995). Both of these approaches have been used to describe the evolution of modern human populations (*e.g.*, Cavalli-Sforza *et al.*, Mccaulay *et al.*, 1999; Kivisild *et al.*, 2002). However, some scholars believe that mutation models lack the sensitivity to detect differences between populations separated by a small period of evolutionary time (Cavalli-Sforza *et al.*, 1996). In these circumstances, models comparing the variation of allele frequencies between sets of populations may more accurately depict biological distances between populations. The relatively small amount of time separating the Kitoi and Serovo-Glazkovo examined in this study suggests that the latter set of biological distance models is better suited for their comparison. Therefore, only these types of biological distance approaches will be considered in further detail. Those interested in exploring how evolutionary models can be applied to aDNA datasets are directed to the review by Kaestle and Horsburgh (2002).

Two biological distance algorithms estimating the variance of allele frequencies in a set of populations are the relationship matrix (R-matrix; Harpending and Jenkins, 1973) and F_{ST} (Wright, 1951; Reynolds *et al.*, 1983). Both of these statistics have been commonly used to compare biological relationships between modern populations. The general formula defining the R-matrix between a set of populations is:

$$Rij = (p_i - p)(p_j - p)/p(1-p)$$

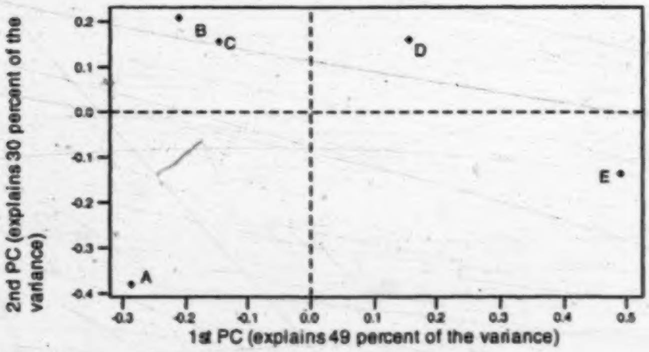
where p_i and p_j represent the frequency of allele p in populations *i* and *j* and p is the mean frequency of allele p in the array of populations considered (Harpending and Jenkins, 1973). The formula for F_{ST} is identical except that the mean frequency of allele p is instead calculated between each pair of populations. The difference between these computations is thought to reflect the models by which populations are observed to diverge (H. Harpending, personal communication, October, 2003). While the R-matrix algorithm is predicted to reflect a population model where geographic distance impacts biological distance between groups, F_{ST} is thought to better reflect a model where two groups diverged from a common ancestor.

Both R-matrix and F_{ST} estimates from allele frequency data can be computed using a number of statistical software packages listed in Appendix 1, including ANTANA for R-matrix analysis (Harpending and Rogers, 1984) and *Arlequin* 2.00 for F_{ST} estimates (Schneider *et al.*, 2000). The numerical output of both R-matrix and F_{ST} can be evaluated in a number of ways. The R-matrix generated from a comparison of alleles for a set of populations is subjected to principal component analysis (PCA) whereby eigenvectors (*i.e.*, principal components) are computed from the covariances of the obtained R-matrix (*e.g.*, Novoradovsky *et al.*, 1993; McComb *et al.*, 1996; Crawford *et al.*, 2002; Rubicz *et al.*, 2002). Principal components (PC) from the R-matrix can then be plotted against each other in a two- or three-dimensional plot which serves as a map of the biological distances between the populations studied. Most commercial statistical software packages with multivariate capabilities will have the capacity to perform PCA. Table 2-2 shows the frequency distribution of six alleles at a single locus for five hypothetical populations. Figure 2-4 shows the two dimensional principal components map derived from the R-matrix of the allele frequencies using MINITAB 13.3. The first two PCs explain 79 % of the total allelic variation observed between the five populations. Populations B and C are observed to be the most similar while A and E are predicted to have the greatest biological distance.

Table 2-2: Allele frequencies for five hypothetical populations

		Population Alleles					
Population	**n**	**a**	**b**	**c**	**d**	**e**	**f**
A	20	0.000	0.083	0.000	0.000	0.583	0.334
B	27	0.333	0.000	0.222	0.000	0.444	0.000
C	25	0.077	0.000	0.385	0.077	0.231	0.230
D	35	0.333	0.095	0.143	0.143	0.095	0.190
E	30	0.158	0.421	0.053	0.158	0.053	0.158

Figure 2-4: Principal component map constructed from R-matrix computations for five hypothetical populations



While the R-matrix algorithm reasonably depicts the similarities in allele frequencies between a set of populations, it is important to note that these relationships have little statistical significance when mtDNA is the only locus evaluated (M. Crawford, personal communication). In order to best depict the degree to which common ancestry is shared between a set of populations, it is necessary that a series of independent loci be evaluated (e.g., Crawford *et al.*, 2002, Rubicz *et al.*, 2002); however, the mtDNA genome is considered to be a single locus and the substitutions defining distinct mtDNA haplogroups are not deemed to be independent alleles. Unfortunately, it is often the case in aDNA studies that mtDNA markers are the only means of evaluating genetic relationships within and between skeletal populations. Thus, interpretations of R-matrix PC maps derived from mtDNA data should be considered as qualitative, rather than quantitative assessments of biological distance.

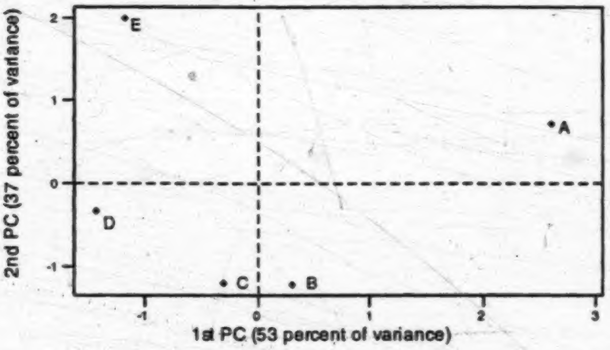
F_{ST} distances are often presented in numerical form within a triangular matrix. Population pairs with F_{ST} values of zero are considered to be biologically undifferentiated while those having F_{ST} values greater than zero have increasing biological distance between them. The F_{ST} distances from the five hypothetical populations considered in the R-matrix example are presented below in Figure 2-5.

Figure 2-5: Biological distances between five hypothetical populations computed from F_{ST}

	A	B	C	D	E
A	0				
B	0.16209	0			
C	0.14874	0.09106	0		
D	0.19747	0.09321	0.06501	0	
E	0.23694	0.20486	0.16161	0.0706	0

F_{ST} distances can also be treated by principal component analysis but the distance matrix needs to be transformed into a similarity (*i.e.* correlation) matrix in order that the relationships between populations are properly represented (Cavalli-Sforza *et al.*, 1996). The PC map derived from the F_{ST} distances are shown in Figure 2-6. Although the values generated for the F_{ST} PC map differ slightly from that obtained from the R-matrix, the proportion of distance between all the populations is observed to be similar.

Figure 2-6: PC map of F_{ST} correlation matrix between five hypothetical populations



One advantage to using F_{ST} to estimate biological distances between an array of populations is that the statistical significance of the observed estimates can be tested when computed using *Arlequin* (Schneider *et al.*, 2000). The generated P values reflect the likelihood that a value larger or equal to the observed F_{ST} estimates can be obtained. In contrast, the statistical significance of an R-matrix analysis cannot be computed directly in the ANTANA software package (Harpending and Rogers, 1984). However, independent tests of significance including the chi-square test of heterogeneity (*e.g.*, Hedrick, 2000: p. 83; Carlyle *et al.*, 2000; Kaestle and Smith, 2001) and exact tests of population differentiation such as the one incorporated into Arlequin (Raymond and Rousset, 1995; cited in Schneider *et al.*, 2000) can be used to assess the likelihood that allelic distributions are significantly different for pairwise population comparisons. Another perceived benefit of the F_{ST} algorithm is that the formula can be derived to estimate the migration rates between two populations and to estimate the time since two populations shared a common maternal ancestor (Weir, 1990; 1995; Schneider *et al.*, 2000; Kaestle and Horsburgh, 2002). However, the tenets of these F_{ST} derivatives suggest that the populations compared need to be contemporaneous for interpretations to be valid; thus, these applications of F_{ST} are not relevant to this study and will not be considered further. Neither R-matrix nor F_{ST} estimates of biological distance have been frequently applied to aDNA data sets. Therefore, inter-population comparisons in this study will consider both these approaches under the assumption that observing concordance between these two statistical estimates will strengthen any derived data interpretations.

Intra-population affinities

Examining the genetic structure within a prehistoric population may provide answers to questions about population size (*e.g.*, Stone and Stoneking, 1998) and provide clues to behaviour promoting or restricting gene flow (*e.g.*, Johnson and Lovell, 1994; Prowse and Lovell, 1996; Rubicz *et al.*, 2002). A valuable statistic to evaluate intra-population biological variation is gene diversity, which is analogous to heterozygosity (*h*) first described by Nei and Roychoudhury, (1974). The gene diversity statistic provides a broad estimate of the genetic variation within a population (Hedrick, 2000). One unbiased estimate of gene diversity for samples of less than fifty individuals is defined by the formula:

$$h = 2n(1-\Sigma x_i^2)/(2n-1)$$

where n is the number of individuals in a sample and Σx^2 is the sum of the squared haplogroup frequencies (Nei and Roychoudhury, 1974; Nei, 1987;

Hedrick, 2000). The variance of the observed gene diversity may be calculated using the following formula:

$$V = 2/2n(2n-1) \{2(2n-2)[\ \Sigma x_i^3 - (\Sigma x_i^2)^2] + \Sigma x_i^2 - (\Sigma x_i^2)^2\}$$

(Formula 8.12; Nei, 1987; Nei and Roychoudhury, 1974). These computations can be calculated in *Arlequin* (Schneider *et al.*, 2000). Nei (1987) recommends that the statistical significance of the variance can then be assessed using a two-tailed t-test.

The gene diversity estimate of Nei and Roychoudhury, (1974) have been used in both modern and ancient DNA studies to make inferences about population size (*e.g.*, Stone and Stoneking, 1998, Schurr *et al.*, 1999; Derenko *et al.*, 2000; 2002). Populations with similar gene diversities are predicted to have similar population sizes. Analogs of this statistic have also been used to explore whether populations have been subjected to pressures promoting genetic drift or population admixture (*e.g.*, McComb *et al.*, 1996; Crawford *et al.*, 2002; Rubicz *et al.*, 2002). Gene diversity estimates from DNA data can be visualised by plotting them against their respective distances from the centroid (*i.e.*, Harpending and Ward, 1982; cited in McComb *et al.*, 1996). Groups with low gene diversity that fall away from the theoretical regression line are predicted to be genetically isolated compared to those with high genetic diversity, found above the regression line that are more likely to have experienced large amounts of gene flow. Using such an approach, gene diversity estimates for modern groups with known population structures can potentially be used as analogs to understand whether prehistoric populations were governed by particular selective pressures.

Gene diversity estimates are typically undertaken using mtDNA RFLP data (*e.g.*, Stone and Stoneking, 1998; Schurr *et al.*, 1999) or protein and nuclear markers (*e.g.*, McComb *et al.*, 1996; Crawford *et al.*, 2002; Rubicz *et al.*, 1998). An analogous measure of mtDNA variation using HVI or HVII sequence data are nucleotide diversity (π; Nei, 1987) which is estimated as:

$$\pi = (1/L)(n/n-1)\ \Sigma\ (1-\Sigma x^2_{ij})$$

where L is the length of the sequence, n is the sample size and xij is the frequency of the i-th nucleotide at site j (e.g., Yao *et al.*, 2002). This study will not consider estimates of π as the mtDNA HVI dataset characterising the Kitoi and Serovo-Glazkovo are to date, incomplete.

Ancient DNA applications

Overview

Ancient DNA studies have crossed many disciplines examining both non-human and human fossil tissue. Krings and colleagues (1997) characterised mtDNA sequences from Neandertal skeletal material to test the hypothesis that *Homo neandertalensis* and *Homo sapiens sapiens* were biologically distinct species. Poinar and colleagues (2001) attempted to retrieve DNA from fossilised coprolites to assess whether the population affinities and diets of ancient North American individuals could be inferred.

The examination of aDNA from human fossil tissue has been applied to explore questions about human identity (*e.g.*, Holland *et al.*, 1993; Gill *et al.*, 1994; Handt *et al.*, 1994; Boles *et al.*, 1995; Ivanov *et al.*, 1996; Jehaes *et al.*, 1998; 2001; Anslinger *et al.*, 2001; Stone *et al.*, 2001), biological distance between ancestral and extant populations (*e.g.*, Stone and Stoneking, 1998; Oota *et al.*, 1999; Shinoda and Kanai, 1999; Benedetto *et al.*, 2000; Carlyle *et al.*, 2000; Wang *et al.*, 2000; Kaestle and Smith, 2001) and the behaviour of ancient groups (*e.g.* Keyser-Tracqui *et al.*, 2003). Several fascinating historical mysteries have been resolved with the use of aDNA techniques. Gill and colleagues (1994) resolved the identity of the Russian royal family who were slaughtered in 1918 during the Bolshevik revolution and buried in a mass grave. Both nuclear and mtDNA markers taken from skeletal remains thought to represent the Romanovs were compared against modern DNA samples from living relatives. The identity of the Tsarina and three of her children was confirmed by matching their mtDNA HVI sequences with that of the Duke of Edinburgh. The identity of Tsar Nicholas II was obscured by a heteroplasmic substitution observed in the mtDNA HVI sequence of the Tsar but not in those of his living maternal relatives. With the subsequent discovery of an identical heteroplasmic HVI substitution in the exhumed remains of the brother of Tsar Nicholas II, (Ivanov *et al.*, 1996), the identity of the Tsar was deemed authentic and the family was laid to rest in the royal tomb.

The long-debated fate of Louis XVII, the son of Louis XVI and Marie-Antoinette, who was imprisoned after the death of his parents, was also resolved with the help of aDNA methods (Jehaes *et al*, 1998; 2001). Many thought that the young Louis-Charles died in prison of tuberculosis. However, others believed that the young man who died in prison was a decoy for the royal prince who escaped captivity and lived out the rest of his life under the name of Naundorff. A 1998 study by Jehaes and colleagues excluded Naundorff as the son of Marie-Antoinette by comparing mtDNA HVI and HVII sequences taken from hair and bone samples from Naundorff's exhumed remains with hair and blood samples from Marie-Antoinette and several maternal relatives. In 2001, Jehaes and others examined mtDNA sequence data from the desiccated heart of the young imprisoned child which had been removed at autopsy and stored in a royal crypt. Independent analyses of mtDNA HVI and HVII sequence data from the heart tissue matched those of three maternal relatives, thus confirming that young child who died in prison in June of 1795 was indeed Louis XVII, the son of the King and Queen of France.

Biological distance Studies

Ancient DNA studies have also explored population affinities between prehistoric human remains and extant populations to test the stability of population structure within a region. Benedetto and colleagues (2000) examined mtDNA sequence data from prehistoric European human remains to assess whether the population structure of western Eurasia changed with the emergence of agriculture. While the Neolithic individuals all harboured mtDNA substitutions shared with modern Europeans, the single Mesolithic individual had a unique mtDNA HVI motif not observed in any contemporary European population. Although the inference of population replacement during the Neolithic is hampered by the small sample size, the observed differences between Mesolithic and Neolithic mtDNA sequences may support the notion that agriculturalists migrated out of the Fertile Crescent and replaced European Mesolithic groups. This hypothesis has the potential to be further enhanced as additional Mesolithic human remains are examined.

Prehistoric and modern North American aboriginal populations have also been compared using aDNA to test hypotheses about the peopling of North America and subsequent population continuity to present times. Stone and Stoneking (1993; 1998) were the first to examine the prehistoric population structure of North America by analysing mtDNA data from a 700 year-old cemetery population representing a single cultural group known as the Oneota. The Oneota were found to share almost all mtDNA lineages with modern North American groups, suggesting that the continental population structure had not dramatically changed over the previous 700 years. The additional Oneotan mtDNA lineage characterised by a HVI motif of 16189, 16223, 16227 and 16278 was subsequently identified in a sample of modern North American Ojibwa as haplogroup X (Brown *et al.*, 1998). Haplogroup G2a, found in East Asian (Kvisvild *et al.*, 2002) and Siberian populations (Derenko *et al.*, 2000; 2003) has a similar HVI motif of 16223, 16227 and 16278; however, G2a individuals are defined by an RFLP motif consisting of combined *DdeI/AluI* site gains at 10394 and 10397 as well as a *CfoI* 7598 site loss. In contrast, haplogroup X is characterised by a loss of the combined *DdeI/AluI* sites and a D*deI* 1715 site loss (Brown *et al.* 1998; Smith *et al.*, 1999). As the Oneota lineages defined by Stone and Stoneking (1998) were not characterised for RFLP markers defining mtDNA haplogroups other than A, B, C and D, it cannot be said with any confidence whether this additional lineage belongs to haplogroup X. Despite this uncertainty, Stone and Stoneking's study (1998) demonstrates that at least five mtDNA haplogroups defined prehistoric North American populations and that prehistoric groups had mtDNA diversities similar to modern aboriginal groups inhabiting North America today.

Subsequent to the study by Stone and Stoneking (1998), two other research groups have examined the biological distance between prehistoric and modern North American populations inhabiting the same region. Carlyle and colleagues (2000) explored the prehistoric population structure of the United States (US) Southwest by comparing mtDNA RFLP data from cemetery populations representative of the Anasazi and Fremont cultures who lived in geographically contiguous regions from approximately 1700 to 1000 years before present (BP) but were thought to be biologically distinct groups. The mtDNA haplogroup distributions of the Fremont (Parr *et al.*, 1996) and the Anasazi

(Carlyle *et al.*, 2000) were found to differ significantly, thus corroborating the notion that these two groups did not exchange genes.

When the biological distances between the Fremont, Anasazi and other prehistoric and modern populations from the southwest US and the Great Basin region of Utah were estimated using the R-matrix algorithm (*i.e.*, Harpending and Jenkins, 1973), the Anasazi and Fremont both clustered with modern Pueblo Southwestern groups. This observation corroborates two distinct hypotheses concerning the fates of both of these extinct cultures. The Anasazi, who inhabited the same region as the modern Pueblo populations, were thought to splinter into smaller groups which then remained in the region (Woodbury, 1979; Sando, 1979; cited in Carlyle *et al.*, 2000). The proximate position of the Anasazi and modern Pueblo groups on the principal component map constructed from the R-matrix estimates suggests that the Anasazi left their genetic signature on the population structure of the region today. The Fremont, who lived to the north of the Anasazi in the eastern Great Basin do not cluster with the modern Great Basin inhabitants sampled in this study. Archaeological and linguistic evidence supports this observation as the disappearance of the Fremont from the archaeological record was thought to coincide with the emergence of a new culture in the Great Basin region which persists to this day (Carlyle *et al.*, 2000; Kaestle and Smith, 2001).

The relationship between prehistoric and modern Great Basin inhabitants was further explored in a study undertaken by Kaestle and Smith (2001). The mtDNA haplogroup distributions of two cemetery populations from western Nevada, sampled by Kaestle and Smith (2001) and the Fremont, analysed by Parr and colleagues (1996) were compared to modern population samples clustered by language and geography. While the Fremont were again observed to cluster with Southwestern groups, thus corroborating the conclusions of Carlyle and others (2000), the western Great Basin groups shared the greatest affinity with modern Californian populations. The lack of population affinity between prehistoric and modern Great Basin groups in these studies (*i.e.*, Carlyle *et al.*, 2000; Kaestle and Smith, 2001) substantiates the idea that the people who inhabit the Great Basin today are not directly descended from prehistoric inhabitants of the region. Furthermore, the significantly different mtDNA haplogroup distributions observed between western and eastern Great Basin groups (Carlyle *et al.,2000*) and their differing population affinities (Kaestle and Smith, 2001) suggest that the prehistoric population structure of this region was quite diverse. The conclusions reached by both of these studies would be further strengthened by analysing additional non-recombining markers (*i.e.*, Y-chromosomal DNA) and sampling additional prehistoric and modern populations to further expand on the fate of the Fremont and their relationship with the Anasazi (Carlyle *et al.*, 2000). However, these studies both demonstrate how hypotheses evaluating the change in the population structure of a region over time, conventionally tested with archaeological and linguistic evidence, are greatly enhanced using biological data retrieved from human fossil material.

Biological affinities studies

The means by which human remains were disposed of in prehistoric communities reflects both the social practices of a group as well as their worldviews (*e.g.*, Binford, 1971; Wason, 1994). Differential mortuary treatment observed between contemporaneous burials in a prehistoric cemetery is generally construed to be a marker of social differentiation (*e.g.* Tainter; 1976; 1978). People buried in graves accompanied by unique artifactual assemblages or other markers of increased energy expenditure are likely to have had higher status in the group than those afforded less elaborate mortuary treatment. While patterns in the archaeological context can reveal many answers about the social structure of a prehistoric population, there are some questions whose answers can only be revealed by evaluating associations between archaeological and biological data. Only with the analysis of biological data would it be possible to unequivocally demonstrate a social system whereby status was inherited rather than achieved in a prehistoric group (*e.g.*, Johnson and Lovell, 1994; Prowse and Lovell, 1996; Hemphill, 1999). Likewise, biological data are the only means of ascertaining who was buried next to whom in a cemetery and whether kinship associations governed post-marital residence patterns (*e.g.* Larsen, 1997: p. 304; Kaestle and Horsburgh, 2002).

Associations between kinship and differential mortuary treatment in cemetery populations have conventionally been explored using continuous and discrete dental and cranial traits to evaluate the biological variation existing between individuals and amongst groups (Larsen, 1997: p 302). Those sharing a suite of specific phenotypic traits are inferred to have greater biological affinity (*i.e.*, be more closely related) than those who have divergent phenotypes. Several studies have used this approach to seek answers for interesting patterns observed in mortuary populations. Studies by Johnson and Lovell (1994) and Prowse and Lovell (1996) revealed a significant association between biological affinity and elite status at the predynastic Egyptian site of Naqada. Both dental and cranial non-metric (*i.e.*, discrete) trait patterning revealed that people interred in the elite cemetery at Naqada had greater biological affinity to each other than to those buried in the two other non-elite cemeteries.

Similarly, Hemphill (1999) used craniometric comparisons to assess whether the elite at the prehistoric Iranian necropolis of Tepe Hissar were derived from the local population. Questions surrounding the population origins of the Tepe Hissar elite arose when unusual artifacts predicted to be of foreign origin were found in association with their burials. Craniometric indices of the Tepe Hissar elite were compared with those from non-elite Tepe Hissar inhabitants as well as individuals who occupied the communities which were considered likely to be the source of the exotic artifacts. The Tepe Hissar elite were found to cluster with other Tepe Hissar inhabitants leading Hemphill (1999) to suggest that the high status Tepe Hissar individuals were derived from the local community and accessed exotic goods through trade networks.

Using phenotypic variation as a marker of biological affinities in mortuary populations has allowed scholars to gain insight into facets of human mortuary behaviour that could not be easily deduced using contextual data. Unfortunately, phenotypic traits do not always accurately depict biological relationships in mortuary populations as these traits are often expressed through the actions of multiple genes and epigenetic mechanisms (Larsen, 1997; p. 303). With the advances gained in aDNA methodologies over the last decade, it is now conceivable to use genotypic markers to assess biological affinities in prehistoric mortuary populations.

Interestingly, questions addressing associations between biological and social behaviour in prehistoric groups have yet to be actively pursued by aDNA scholars. Only a couple of exceptions have been noted to date. Shinoda and Kanai (1999) examined aDNA HVI sequence data retrieved from 29 individuals who had been deposited in a Japanese midden dating to 2500 BC. Two mtDNA lineages were found to represent 76 % of the total mtDNA variation observed at the site, thus leading the authors to hypothesise that the midden represented a mass burial of closely related individuals. The results of Shinoda and Kanai (1999) agreed with a previous odontometric study that also identified the presence of two major family groups (Matsumura and Nishimoto, 1996; cited in Shinoda and Kanai, 1999). However, seven additional mtDNA lineages retrieved from people interred in the midden suggest that people other than maternal relatives were buried together. Shinoda and Kanai (1999) note that the analysis of additional DNA markers will be necessary to fully understand the relationships between all individuals interred in this mass burial. Regrettably, this study did not discuss whether the observed midden burial was typical for Jomon-era necropoli. If this interment had been recognised as unusual Jomon mortuary behaviour, comprehensive analysis of the archaeological context may have yielded important additional evidence about the behaviour of a prehistoric group.

The only other biological affinities study of a cemetery population identified using DNA markers was recently undertaken by Keyser-Tracqui and colleagues (2003) who examined a Northern Mongolian cemetery known as Egyin Gol, dating to the 3rd century B.C. The authors created DNA profiles for over 50 individuals from STR, Y-chromosomal and mtDNA markers and evaluated these profiles in the context of spatial data to assess whether burials were patterned as a reflection of kinship. The earliest sector of Egyin Gol was found to have the fewest number of related individuals; only one parent-child set of burials was observed here. Interestingly, this part of the cemetery held the sole high status burial noted at Egyin Gol and surrounding this burial were a collection of double interments. The individual in the elite burial was male and did not share any obvious biological affinity with any other person interred in this part of the cemetery. Keyser-Tracqui and colleagues (2003) suggest that this patterning was not uncommon for high status burials from this era and cite ethnographic and archaeological evidence (Francfort *et al.*, 2000) to support their claim. In the remainder of the cemetery, many familial relationships were observed between people buried proximal to each other including parent-child and sibling-sibling pairs. A distinct cluster of male burials defined by a single Y-chromosomal lineage was also identified; various combinations of mtDNA and STR profiles within this cluster suggest that membership in this group extended beyond father-son or brother-brother pairs. The authors did not attempt to compare biological profiles between individuals within double interments to understand if kinship impacted this type of mortuary behaviour. This was largely a qualitative paper and would have been greatly enhanced if greater consideration had been paid to associations between the genetic and contextual data. However, the DNA data retrieved from Egyin Gol are valuable to this study, due to the proximity of this cemetery to the two cemeteries examined in the following papers and will be discussed again in the context of Cis-Baikal population affinities.

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Chapter 3: Verifying the Authenticity of mtDNA data from Curated Human Skeletal Remains

Note of co–authorship

Mr. Mark Hicks of the Molecular Diagnostics Laboratory contributed to this work in that he was responsible for the optimising the analysis of the results discussed in this study.

Introduction

Ancient DNA (aDNA) analysis in mortuary archaeology is emerging as a valuable tool in revealing the population structure of archaic peoples (*e.g.*, Stone and Stoneking, 1998; Kaestle and Horsburgh, 2002; Mooder *et al.*, 2003). Ideally, skeletal samples from mortuary contexts should be earmarked for aDNA analysis at the time of excavation. However, aDNA analysis has also been attempted using skeletal collections curated after excavation (*e.g.*, Stone and Stoneking, 1998), making it necessary to assess whether exogenous DNA transferred through handling of skeletal material can be effectively recognised. Many studies have discussed criteria to identify and circumvent contamination introduced during aDNA analysis (*e.g.*, Hummel and Hermann, 1994; Jehaes *et al.*, 1998; Eshlemen and Smith, 2001). However, fewer studies have acknowledged the impact that exogenous DNA introduced through handling has on the retrieval of authentic DNA data (*e.g.*, Handt *et al.*, 1994, 1996; Richards *et al.*, 1995; Kolman and Tuross, 2000). The purpose of this paper is twofold: first, to examine whether modern DNA contamination of archaeological bone material produces consistent, recognisable patterns and second, to propose criteria to ensure the authenticity of mitochondrial DNA (mtDNA) sequence data reported from studies using curated skeletal remains.

Materials and Methods

Samples

The skeletal population discussed in this study was excavated from the Lokomotiv cemetery site in the southern Siberian city of Irkutsk. Lokomotiv is considered to be the largest Neolithic cemetery in north Asia and was excavated by Russian archaeologists from Irkutsk State University (ISU) between 1980 and 1997 (Bazaliiskiy and Savelyev, 2003). Ancient DNA analysis of the Lokomotiv collection was initiated in 1998 as part of the Baikal Archaeology Project (BAP), a large-scale interdisciplinary effort attempting to characterise the lives of Neolithic Siberian hunter-gatherers (Weber *et al.*, 2002). Since the Lokomotiv skeletal remains had been curated in the department of Archaeology and Ethnography at ISU since excavation, it became necessary to assess whether exogenous DNA contributed through handling impacted our ability to retrieve authentic DNA signal. Available ^{14}C dates for the individuals discussed in this study range from 6660 to 6440 years before present (BP). Overall, the samples are macroscopically well preserved having been interred in graves composed of reddish-brown loam at depths generally exceeding 50 cm. Although this skeletal collection was not known to be used for osteological instruction (V. Bazaliiskii, personal communication), the chain of custody for these samples has proved challenging to trace. Both osteological (Link, 1996) and archaeological studies (Mamonova and Bazaliiskiy, 1986) have been undertaken using the Lokomotiv collection.

Specimen handling

To assess the effects of DNA transfer by handling, ten samples from five subadult individuals interred at Lokomotiv were selected for analysis. These subadult samples were initially selected for DNA sexing using the amelogenin locus (*e.g.*, Manucci *et al.*, 1994) However, it was deemed necessary to verify the authenticity of the retrieved DNA prior to performing DNA sexing. Duplicate long bone sections (femur, humerus, or tibia) were taken from three of the individuals while two sections of vertebrae were taken from the remaining two individuals. A volunteer (LM) was instructed to handle the selected bone samples in a manner similar to a physical anthropologist making typical osteological observations. This volunteer was never involved in the analysis of any samples included in our study. The samples were then transferred to plastic bags and analysed in the Human Identification Laboratory for Archaeology (HILA). At the same time, a blood sample was collected from LM for mtDNA sequencing. A blood sample was also collected from the Dr. David Link (DL), the physical anthropologist who conducted the osteological examination of the Lokomotiv skeletal material during 1995 and 1996.

Specimen preparation

The handled samples were prepared for extraction in two series. For the first series of samples, 1 to 2 mm of bone surface were removed using a sterile scalpel to eliminate the transferred DNA and other potential contaminants from the surface of the bone (*e.g.*, Stone and Stoneking, 1998). The scraped bones were then briefly immersed in a 10 % bleach solution[1] (1-5 minutes, depending on the observed porosity of the sample) and rinsed in sterile distilled water (*e.g.*, Prince and Andrus; 1992; Richards *et al.*, 1995). The samples were transferred to individual sterile, plastic containers and exposed to UV light (254 nm) at a distance of 5 cm for a minimum of one hour (*e.g.*, Ou *et al.*, 1991). To test whether surface removal of bone was a sufficient to eliminate exogenous DNA contamination, the second series of bones were taken only through the bleach and UV irradiation steps. Following UV irradiation, samples were dried overnight. When dry, samples were immersed in liquid nitrogen (within their sterile containers) for a minimum of 30 minutes and subsequently pulverized using sterile mortars and pestles. All sample preparation and subsequent pre-

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[1] 10% v/v solution of industrial strength bleach containing 12%w/v sodium hypochlorite.

PCR analytical manipulations were carried out within a laminar flow hood that was completely wiped with undiluted bleach[2] prior to each use. All equipment and supplies used within the laminar flow hood were rendered DNA-free through autoclaving (mortars and pestles, extraction and wash buffers), undiluted bleach treatment (pipettors, tip boxes, racks) or UV irradiation (most reagents).

DNA extraction

All samples from both the fully treated and partially treated series were extracted in duplicate using the guanidium thiocyanate protocol originally proposed by Boom and colleagues (1990) with the following modifications: the initial 65 °C incubation in extraction buffer was extended from 1 hour to a minimum of 16 hours and incubation of the silica binding step was increased from 20 minutes to two hours. In the final silica elution step, 100 µL of sterile, deionised water was added to the dried silica pellet and incubated in a 56 °C water bath for one hour. Two blanks were extracted in tandem with the samples. The eluted samples were then stored at –20 °C until analysis.

PCR amplification and DNA sequencing

A 176 base pair (bp) region of the mtDNA hypervariable region I (HVI) from np 16191 to np 16367 was chosen based on the number of informative Asian-specific substitutions known to exist in this part of the mtDNA genome (*e.g.*, Schurr *et al.*, 1999; Kivisild *et al.*, 2002). The length of the sequenced region was limited to 176 bp as we had previously observed other samples of comparable antiquity to fail to amplify with primers flanking regions greater than 180 bp (Mooder *et al.*, 2003). The primary PCR was undertaken in a 50 µL reaction mix using 1.5 mM $MgCl_2$ (Invitrogen), 16 µg BSA (NEB), 0.2 mM dNTPs, (Invitrogen), 20 pmol each primer L 16211 5′-CCCATGCTTACAAGCAAGTAC and H 16346 5′-GGGACGAGAAGGGATTTGAC (DNA synthesis lab, University of Alberta) and 1.25 U of Platinum Taq Polymerase (Invitrogen). PCR was performed in a PTC 125 Minicycler (MJ Research, Boston, MA) and reaction conditions included an initial soak at 95 °C for 2 min, followed by 50 cycles of 95 °C for 1 min, 54 °C for 1 min 30s and 72 °C for 1 min. All pre- and post-PCR manipulations were carried out in physically separated rooms. Following PCR, all reactions were screened by electrophoresing 10 µL of PCR product on 10 % polyacrylamide gels. The PCR products were purified using the QuickStep 2 PCR Purification kit (Edge Biosystems) and sequencing of 75 ng of template was undertaken for both the H and L strands using an ABI 377 sequencer and the BigDye terminator package (Applied Biosystems). All sequence data were aligned manually and substitutions deviating from the Cambridge reference sequence (Anderson *et al*, 1981) were noted.

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[2] industrial strength bleach containing 12% w/v sodium hypochlorite.

Results and Discussion

The summarised mtDNA HVI data as well as a subset of the raw sequence generated from this study are shown in Appendix 2a and 2b respectively; each sequence is derived from an independent extract. Of the 17 extracts generating mtDNA sequence data, the prevailing sequence is characterised by substitutions deviating from the Cambridge reference sequence at 16224 and 16311. This is a European mtDNA sequence which corresponds to haplogroup K and matches that of the osteologist. Of the 14 samples with the 16224 and 16311 motif, seven were heteroplasmic at 16224, suggesting that a heterogeneous mixture of mtDNA molecules was present in the extracts. One of these sequences was also heteroplasmic for C/T at 16298 which may reflect contamination from the analyst who is defined by a C-T substitution at this position.

Eight extracts showed an additional C-T transition at 16320, a substitution not present in the sequence of the osteologist and is not characteristic of any known Asian mtDNA haplogroup motif (*e.g.*, Schurr and Wallace, 1998). Another four extracts were heteroplasmic for C/T at 16320. That the 16320 substitution is not common to any Asian haplogroup and found in extracts from all five individuals suggests that this substitution is not likely to be endogenous. Interestingly, the 16320 C-T transition is found in the mtDNA sequence of LM, the experimental handler; suggesting that this substitution arose through the phenomenon known as jumping PCR. Jumping PCR has been discussed in other aDNA studies (Paabo *et al.*, 1990, Handt *et al.*, 1994) and occurs when a partially extended amplification product encounters DNA template damage and disassociates from the DNA polymerase complex. The partially extended amplification product may then anneal to a different mtDNA copy where extension continues. The resulting sequence data will be a hybrid of the mtDNA molecules present in a PCR, reflecting what we observe here.

The two other sequences retrieved included an Asian sequence with an HVI substitution characteristic of mtDNA haplogroup A (16223T and 16290T), and a sample that deviates from the Cambridge sequence at one position (16256T). Three extracts failed to produce any sequence from multiple amplification attempts.

We are perplexed to observe that the prevailing mtDNA sequence from our experimental data represents the osteologist who handled these bones eight years prior to our analysis, rather than the individual who handled the bones most recently. However, both environmental circumstances and curation challenges may have influenced this finding. The volunteer conducted this experiment in a modern air conditioned laboratory, washed his hands prior to handling any of the specimens and manipulated each specimen only once over a period of several minutes. In contrast, the osteologist handled these specimens while sorting commingled skeletal material during the summer months in a building lacking air conditioning or accessible hand washing facilities. Given that the osteologist was likely to have handled these samples multiple times, one might intuitively think this would increase the likelihood of DNA transfer to the bones. However, empirical experiments examining DNA transfer suggest that DNA transfer is instantaneous and that the number of handling events does not increase the quality of the DNA signal retrieved (Wickenheiser, 2002). An alternative explanation considers that more DNA was transferred to these specimens by the osteologist than by the volunteer handler. Such a scenario is supported by the observations of Lowe and colleagues (2003) who conducted experiments to observe whether uniform quantities of DNA were transferred to inanimate objects by different individuals. Interestingly, a complete DNA profile could be retrieved from objects handled by certain individuals for as little as 10 seconds, whereas others left little DNA at all.

The issue of hand washing appears to be secondary to that of the shedding ability of an individual; while exceptional DNA shedders may transfer DNA immediately after hand washing, it may take poor shedders up to six hours post-hand washing to transfer a measurable quantity of DNA (Lowe *et al.*, 2002). Thus, while hand washing (or the lack thereof) may not have influenced the transfer of DNA to the specimens considered in our experiment, our observations suggest that that the osteologist was a more efficient DNA shedder than the experimental handler.

Further to the issues of DNA transfer it appears that the depositional environment of the skeletal remains examined in this experiment influenced the preservation of the specimens and thus, our ability to retrieve an endogenous DNA signal. Individuals at the Lokomotiv cemetery were interred in single, double, triple or communal burials (greater than three individuals). Interestingly, no appreciable differences in the dimensions of the burial pits were observed between all grave types (Bazaliiskiy and Savelyev, 2003). Consequently, individuals in the communal interments were buried layered upon each other in pairs. We believe that the compact arrangement of bodies in the communal graves may have accelerated diagenesis (*i.e.*, postmortem decomposition), thus degrading the DNA intrinsic to the bone. Four of the five subadults selected for this study were interred in graves containing more than four individuals; the fifth was excavated from a double burial.

The association between endogenous DNA retrieval and grave type is supported by the mtDNA data produced in our broader Siberian mtDNA study (Mooder *et al.*, 2003; Mooder *et al.*, in preparation). Whereas we have obtained unequivocal Asian mtDNA RFLP and HVI sequence data from most individuals in single, double and triple interments at Lokomotiv, mtDNA HVI data generated from individuals in the multiple burials have predominately been exogenous mtDNA sequences, presumably derived from handling (Chi-square, p= 0.05, SYSTAT 10.2). As contaminating mtDNA sequences from multiple graves have been retrieved from both adult and subadult individuals, it seems more likely that the depositional environment rather than age-associated diagenetic change (*e.g.*, Stojanowski *et al.*, 2002) is effecting endogenous DNA retrieval. It has also been proposed that DNA from compact bone is better protected from post-mortem assault than DNA in cancellous bone (Henderson, 1987). However, both the long bones and vertebrae analysed in this study produced contaminating mtDNA sequence data suggesting that all skeletal elements from multiple graves at Lokomotiv are similarly influenced by handling.

Our experimental observations suggest that the conventional aDNA approaches of surface removal, bleach treatment and UV irradiation (*e.g.*, Kaestle and Horsburgh, 2002) do not reliably remove exogenous DNA transferred to bones through extensive handling. Interestingly, we observe no difference in the quality of the sequences generated from the fully or partially treated sample series, suggesting that surface removal is no more effective in removing exogenous DNA contamination than bleach treatment and UV irradiation alone. Our data supports studies by Kolman and Tuross (2000) and Richards and colleagues (1995) who found that while bleach treatment of bone could reduce interference from contaminating DNA, exogenous DNA could not be removed entirely. Richards and colleagues (1995) were able to remove human DNA from animal bone surfaces through shot blasting but this approach may only be appropriate for the most robust of osteological samples. Clearly, if DNA analysis of curated skeletal remains is to continue, improved methods to destroy contaminating DNA need to be identified.

Given the likelihood that DNA extracts from curated skeletal remains will contain contaminating DNA sequences, investigators need to carefully evaluate the authenticity of the aDNA data retrieved. Many studies have proposed criteria to verify the authenticity of aDNA sequence data (*e.g.*, Richards *et al.*, 1995; Handt *et al.*, 1996; Kolman and Tuross, 2000; Cooper and Poinar, 2000) and while all these criteria have merit, they do not necessarily allow the analyst to discriminate exogenous DNA from authentic sequence (Kolman and Tuross, 2000; Kaestle and Horsburgh, 2002). For example, the intent of DNA quantification through competitive PCR is to enumerate the number of endogenous DNA molecules remaining in a sample (*e.g.*, Handt *et al.*, 1994).

However, Kolman and Tuross (2000) demonstrated through quantitative PCR that contaminated and uncontaminated extracts contained similar quantities of DNA. Therefore, establishing that a DNA extract contains a sufficient number of mtDNA molecules of a size appropriate to aDNA, does not guarantee that the sequence data are authentic (Kolman and Tuross, 2000; Kaestle and Horsburgh, 2002).

Similarly, cloning of PCR products has been proposed as the gold standard to determine aDNA authenticity (*e.g.*, Cooper and Poinar, 2000). While cloning allows the analyst to characterise the composition of a PCR product and detects the presence of exogenous DNA with greater resolution, our results suggest direct sequencing of PCR products is similarly effective in detecting interfering, exogenous DNA sequences and is far less expensive and laborious.

In contrast, the use of sequence reproducibility as an authenticity criterion will help identify exogenous DNA sequence data, when present. For example, we observe that no individuals in our experiment generate reproducible mtDNA sequences from all four independent extracts (we did not attempt to verify intra-extract reproducibility). In contrast, HVI sequencing performed on other individuals from Lokomotiv has generated reproducible sequence data from duplicate extractions over multiple amplification events (Mooder *et al.*, in preparation). Ensuring that sequence data are phylogenetically sound also helps to discriminate between endogenous and exogenous mtDNA sequences (*e.g.*, Stone and Stoneking, 1998; Kolman and Tuross, 2000) and is illustrated by our data. First, by understanding what mtDNA polymorphisms are likely to be found in our study population (*i.e.*, Asian), we are able to recognise that the prevailing European sequence produced in our analysis is suspicious. The presence of the 16320 substitution in over half of our sequences is also questionable as this substitution is not characteristic of the mtDNA haplogroup K motif; lending additional support that these sequences are not authentic.

We propose that heteroplasmy can also serve as an effective marker of exogenous DNA contamination. Although heteroplasmy has been observed in aDNA studies using mtDNA, (*e.g.*, Gill *et al.*, 1994; Ivanov *et al.*, 1996; Jehaes *et al.*, 1998), the reproducibility of the heteroplasmic substitution ensures its authenticity. While our experiment produced heteroplasmic mtDNA sequences, the heteroplasmy was not uniformly reproducible between extracts belonging to the same individual. The only exception was individual 161 who is heteroplasmic at position np 16224 in the three mtDNA sequences generated. However, given the same position is heteroplasmic in three of the four other individuals at varying frequencies, it is unlikely that the heteroplasmy reflects an authentic sequence. Therefore, non-reproducible, prevalent, heteroplasmy should be considered highly indicative of contamination, unless it can be proven otherwise (Kolman and Tuross, 2000).

When considering the analysis of DNA from curated skeletal remains, it is essential that the chain of custody of the samples be traced and reference samples collected from any individual known to have handled the skeletal material. When working with skeletal remains derived from the same population as the investigators (*i.e.*, European, Asian or African), reference samples from all people involved in the analysis are crucial to verify the authenticity of the mtDNA sequence data retrieved. Although the specific excavation dates of each burial at Lokomotiv are difficult to trace, the skeletal remains examined in this study were curated at ISU for as long as twenty years prior to our analysis. While we have a reference sample from the individual who conducted the osteological examination of the Lokomotiv collection, there are certain to be other individuals who transferred DNA to these bones. As these skeletal remains are expected to possess Asian-specific mtDNA polymorphisms, any E[illegible]pean mtDNA sequences detected in our analysis have been rejected as contamination (Mooder *et al.*, unpublished data). Given that no individuals possessing Asian mtDNA polymorphisms are known to have been associated with the excavation or curation of the Lokomotiv collection, (D. Link, personal communication), we are confident that the Asian mtDNA sequence data we have retrieved from Lokomotiv are authentic.

In addition to tracing the curation history of skeletal remains bound for aDNA analysis, we propose that curated bone samples should be screened for exogenous contamination by mtDNA HVI sequencing before any other genetic manipulations (*e.g.*, DNA sexing or mtDNA RFLP analysis) are undertaken. If exogenous mtDNA sequences are detected from multiple, independent extracts, the likelihood of obtaining endogenous, authentic mtDNA data are small. It is also sensible to consider only aDNA data with concordant RFLP and HVI substitutions to be authentic (e.g., Yao *et al.*, 2003), The successful retrieval of endogenous, authentic mtDNA sequence data from bone may also be improved if DNA is extracted from a number of different bone samples from each individual under study. Bone elements typically subjected to less scrutiny by osteologists (*e.g.*, thoracic vertebra, rather than long bones) might also improve the chance of yielding endogenous DNA.

Summary

Our results suggest that DNA transfer through handling has a profound effect on the retrieval of endogenous mtDNA sequences from curated skeletal remains. The conventional aDNA protocols for sample decontamination are insufficient for removing exogenous DNA entirely and need to be revisited. Poorly preserved bone samples that are handled are most likely to produce exogenous mtDNA sequences, while bones in good preservation may contain sufficient endogenous DNA molecules to produce identifiable, authentic mtDNA data. Although exogenous contamination of skeletal material does not necessarily produce uniform patterns when DNA is analysed, there are recognisable discrepancies that allow an investigator to question the authenticity of mtDNA data retrieved. The presence of non-reproducible heteroplasmy and polymorphisms peripheral to those RFLP and HVI substitutions expected of a given mtDNA haplogroup motif are both evidence that the mtDNA data are not authentic. In addition, mtDNA HIV sequence data that are not characteristic of the population under study should also be considered suspect.

If at all possible, skeletal remains destined for DNA analysis should be committed at the time of excavation and always handled wearing gloves. In circumstances where DNA analysis of curated human remains is desired, it will be necessary to obtain DNA reference samples from any individual who handled the samples. We advocate that mtDNA sequencing should be undertaken on all handled bone elements prior to any other aDNA manipulations. If the retrieved mtDNA data reflect the sequence of a reference sample (*i.e.*, someone who handled the bones), it is likely that endogenous, authentic mtDNA sequence data are not recoverable. Furthermore, mtDNA data obtained from curated skeletal remains should be reproducible from duplicate extracts taken from a minimum of two discrete samples. It is crucial for those undertaking aDNA analysis of human skeletal remains to acknowledge and recognise how DNA transferred through handling impacts the likelihood of retrieving authentic aDNA data.

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Chapter 4: The Matrilineal Population Structure of Prehistoric Siberia: An Examination of Biological Continuity in Neolithic Cis-Baikal

Introduction

While archaeological evidence definitively supports the inhabitation of the Lake Baikal region in the southwest of Eastern Siberia since the Upper Palaeolithic (Okladnikov, 1959; 1964; Alekseev, 1998); little is known about the population history of the region and whether biological continuity can be demonstrated from the Palaeolithic through to contemporary times. Lithic evidence from excavations at the Upper Palaeolithic sites of Mal'ta and Buret' in the Angara river basin suggests that the earliest inhabitants of the Cis-Baikal region (*i.e.*, north of Lake Baikal) had a material culture similar to those of contemporaneous Eastern European groups (Okladnikov, 1959; 1964). Although it is well established that the Altai region of Western Siberia has been the site of notable admixture between East Asian and West Eurasian populations since the Upper Palaeolithic (Derenko *et al.*, 2002a; 2002b; 2003), limited osteological evidence (Turner, 1987; Ishida and Dodo, 1996) and Palaeolithic artistic representations (Debets, 1951; cited in Okladnikov, 1959; Okladnikov, 1964) suggest that the original inhabitants of Eastern Siberia including Cis-Baikal, were of Asian origin. The abundance of both habitation and cemetery sites along the major river basins throughout the south-western part of East Siberia speak to its rich population history. Although many of these sites have been excavated by Russian archaeologists (*i.e.*, Gerasimov, 1958; Okladnikov, 1959; 1964), very few skeletal collections found within have been characterised extensively. However, understanding the biological character of these past Siberian groups provides an opportunity to piece together the population history of the region and to better understand how these past peoples contributed to the population structure of Siberia as it is today.

This study provides the opportunity to illuminate the population history of prehistoric Siberia through the examination of two Cis-Baikal Neolithic cemetery populations located on the Angara river, downstream from Lake Baikal. The use of the two cemeteries, known as Lokomotiv and Ust'-Ida, flank an 800-year gap in the archaeological record that is thought to represent a biocultural hiatus in the Cis-Baikal region (Weber, 1995; Weber *et al.*, 2002). Differences in the material culture, burial practices and subsistence strategies as observed in the mortuary record suggest that the two populations were dissimilar. Russian anthropologists believe the groups using Lokomotiv and Ust'-Ida were biologically discrete based on observed variation of cranial traits (Gerasimov, Maumanova, 1991; cited in Weber, 1995).

The Lokomotiv cemetery (**Figure 4-1**), located in the current-day city of Irkutsk at the confluence of the Irkut and Angara rivers, was used by a pre-hiatus population known as the Kitoi. The Kitoi are characterised by a subsistence strategy heavily reliant on fishing and had unique mortuary rituals including the widespread use of red ochre and decapitation in burials (Bazaliiskii and Savelyev, 2003). Weber and colleagues (2002) proposed that the Kitoi were a population in decline towards the latter half of their existence due to stresses imposed by, among other things, a selective subsistence strategy and social isolation. The Kitoi culture disappeared from the archaeological record after approximately 6000 years before present (BP) and for most of the next 800 years, no evidence of cemeteries can be found in the Cis-Baikal region. Around 5100 B.P., a different culture emerged in Cis-Baikal as evidenced by the mortuary record at Ust'-Ida, located approximately 100 km downstream of Lokomotiv at the mouth of the Ida river.

The graves at Ust'-Ida (**Figure 4-1**) represent two groups known as the Serovo and Glazkovo. Although Serovo and Glazkovo mortuary rituals vary slightly, scholars believe that their other cultural behaviour, such as mobility patterns and subsistence strategies are similar enough to warrant their treatment as a single group (Weber, 1995; Weber *et al.*, 2002). The Serovo-Glazkovo are observed to differ from the Kitoi both in the associated material culture found within the burials as well as their mortuary behaviour, mobility patterns and subsistence strategies. The Serovo and Glazkovo used fire as a part of mortuary ritual, although traces of ochre were also found in the occasional grave (Tiutrin and Bazaliiskii, 1996; Weber *et al.*, 2002). Furthermore, tool kits and stable isotope data retrieved from Serovo and Glazkovo skeletal material suggest that they primarily hunted for food (Weber *et al.*, 2002). Investigators have proposed that the Serovo-Glazkovo community using Ust-Ida was larger and healthier than the Kitoi due to the increased incidence of osteoarthritis and enamel hypoplasia observed in the Kitoi and the abundance of Serovo-Glazkovo subadults in the mortuary record (Link, 1996; Weber *et al.*, 2002).

Figure 4-1: Location of Neolithic Cis-Baikal cemeteries analysed in this study

Although the health, demography and subsistence strategies of the Kitoi and Serovo-Glazkovo have been investigated relatively well (Link, 1996;

Lieverse, 1999; Link, 1999; Katzenberg and Weber, 1999; Weber *et al.*, 2002), a comprehensive biological affinities analysis has never been published on these skeletal collections. There is however, substantial value in estimating the biological (*i.e.*, genetic) distance between the Kitoi and the Serovo-Glazkovo as such an approach will allow the biological discontinuity hypothesis in Neolithic Cis-Baikal to be more conclusively tested. Biological affinities analyses of skeletal populations have traditionally been performed by observing variation in dental, cranial and post-cranial metric and non-metric traits (*e.g.*, Johnson and Lovell, 1994; Prowse and Lovell, 1995; 1996; Hemphill, 1999). However, continued advances in ancient DNA analysis have made it possible to catalogue variation in prehistoric cemetery populations using genotypic rather than phenotypic data (*e.g.*, Stone and Stoneking, 1998; Carlyle *et al.*, 2000; Kaestle and Smith, 2001; Keyser-Tracqui *et al.*, 2003). It is generally thought that biological distances between groups are better estimated using genotypic traits as phenotypes are often expressed through the action of multiple genes or other epigenetic influences (Larsen, 1997; Gelehrter *et al.*, 1998).

The principal objective of this paper is to explore whether the biological distance between the Neolithic Kitoi and Serovo-Glazkovo can be estimated using mtDNA polymorphism data. The high copy number of mtDNA in bone tissue facilitates its retrieval from human skeletal remains (Giles *et al.*, 1980; Wallace *et al.*, 1987). With the retrieval of sufficient, authentic mtDNA polymorphic data to characterise both the Kitoi and Serovo-Glazkovo, it may be possible to provide evidence to support a significant depopulation event in the Cis-Baikal region during the Middle Neolithic followed by the emergence of a new group in the same area almost a millennium later.

For years, knowledge of modern Siberian mtDNA structure was limited to the distributions of haplogroups A, B, C, and D that were used to explore hypotheses concerning the peopling of the Americas (Schurr *et al.*, 1992; Torroni *et al.*, 1993a; 1993b). When scholars began to explore variation in the mtDNA hypervariable (HV) regions, it became evident that the mtDNA composition of modern Siberian populations was highly diverse (Shields *et al.*, 1993; Stariskovskya *et al.*, 1998; Schurr *et al.*, 1999). Since then, both RFLP and HVI data has been comprehensively described for many modern Siberian groups (Derenko *et al.*, 2000; 2001a; 2002b; 2002a; 2002b; 2003).

The recent enhancement of the Siberian mtDNA datasets has allowed relationships between modern Siberian populations to be characterised with greater sensitivity. It has recently been established that the matrilineal structure of south Siberian populations are highly differentiated by geography (Derenko *et al.*, 2003), which is consistent with the tenet that groups exchange genes with their neighbours (Cavalii-Sforza *et al.*, 1996; Hedrick, 2000). Thus, groups living in the regions proximate to Cis-Baikal are expected to share a greater degree of biological affinity with each other than with populations outside the region. Several aDNA studies have demonstrated the continuity of mtDNA population structure of North America through thousands of years (*e.g.*, Carlyle *et al.*, 2000; Kaestle and Smith, 2000). There is contention about whether groups inhabiting the Cis-Baikal region today are descendents of those who inhabited the region during the Neolithic (Lopatin, 1940; Okladnikov, 1964; Naumova and Rychkov, 1998). By exploring which modern and Neolithic Siberians share mtDNA affinities, it may be possible to determine if the Kitoi or Serovo-Glazkovo contributed to the matrilineal population structure of the Cis-Baikal region today.

Materials and Methods

Neolithic population samples

The skeletal remains representing the Kitoi and Serovo-Glazkovo analysed in this study were excavated from the Lokomotiv and Ust'-Ida cemeteries by Russian archaeologists from Irkutsk State University (ISU) during the last two decades of the 20th century (Tiutrin and Bazaliiskii, 1986; Bazaliiski, 2003; Bazaliiskii and Savelyev, 2003). Lokomotiv is considered the largest Neolithic cemetery in North Asia and was first discovered during construction of the Trans-Siberian Railway in the late 1800s (Bazaliiskii and Savelyev, 2003). Non-calibrated radiocarbon dates (Isotrace, University of Toronto) from Lokomotiv suggest that this cemetery was used from approximately 7250 to 6040 BP; these dates correspond to a period between 6125 to 4885 B.C. when calibrated using the methodology of Stuiver and others (1998; R.Buekens, personal communication). In total, 70 graves containing 124 individuals were excavated from Lokomotiv and the retrieved skeletal remains were curated in the Department of Archaeology and Ethnography at ISU. Multiple cervical or thoracic vertebrae were sampled from 40 Lokomotiv individuals in 1995 for subsequent DNA analysis to be carried out at the University of Alberta.

The Ust'-Ida cemetery was excavated after a dam on the Angara river downstream of the cemetery flooded in 1986 causing 11 graves to erode out of the bank (Tiutrin and Bazaliiskii, 1996). Uncalibrated radiocarbon dates obtained from skeletal remains at Ust-Ida place the use of this cemetery between approximately 4960 and 3590 BP; these dates correspond to a period between 3710 and 2020 B.C when calibrated using the methodologies of Stuiver and others (1998; R. Buekens personal communication). Between 1987 and 1996, 59 graves containing 64 individuals were excavated; of these, multiple cervical and thoracic vertebrae were sampled for DNA analysis from 42 individuals. Based on differences in body orientation, two grave typologies have been identified at Ust-Ida (Titurin and Bazaliiski, 1996). The first is the Serovo type (n= 37) in which burials are found oriented in an extended supine position with the heads pointing to the south. The second is the Glazkovo type (n=12) in which burials are either found in an extended supine or flexed body position with the heads oriented to the north. While there is little overlap between the ^{14}C dates for the Serovo and Glazkovo graves at Ust-Ida (A. Weber, personal communication), these groups are treated as a single community for a number of reasons. Firstly, based on similarities in osteological traits and material culture, Russian anthropologists (*i.e.*, Gerasimov, 1955; Maumanova,1983; Gerasimova, 1991; cited in Weber, 1995) believe that the Serovo and Glazkovo are biologically and culturally continuous. Secondly, many Serovo-Glazkovo cemeteries are known to exist throughout the Cis-Baikal region where the dates corresponding to the use of both grave types are observed to overlap (Weber, 1995; Weber *et al.*, 2002). Finally, the Glazkovo sample at Ust-Ida is too small to make any meaningful inferences about how they differ from the Serovo.

Overall, based on macroscopic examination, the skeletal remains from both cemeteries appear to be well preserved. The grave pits at Lokomotiv were generally dug to depths exceeding 50cm into the lower levels of reddish brown loam corresponding to the Holocene climatic optimum *i.e.*, the Atlantic period (Bazaliiski, 2003). Many of the grave pits at Ust-Ida were dug to the upper levels of the reddish brown loam and backfilled with stone slabs and cobbles. The climate of the Lake Baikal region is continental with warm summers, cold winters and limited precipitation; regions of discontinuous permafrost have also been noted in the area around Lake Baikal (Weber *et al.*, 2002) but not in the Angara valley. The combination of neutral soil pH, good soil drainage and cool temperatures favours the preservation of DNA within the osteological material (Rogan and Salvo, 1990, Eglington and Logan, 1991; Geigl, 2002).

Specimen preparation

To remove any exogenous DNA contaminating the skeletal material, all samples were prepared for analysis first by removing several millimetres of the bone surface with a sterile scalpel, followed by immersion in a 10 % bleach solution[3] (with time dependent on the observed porosity of the sample). The samples were then briefly rinsed in sterile, distilled water and exposed to UV irradiation at 254 nm for a minimum of an hour within closed, sterile containers. The samples (within their sterile containers) were then immersed in liquid nitrogen for 20 to 60 minutes (depending on the sample size) and crushed with a sterile mortar and pestle. The pulverised samples were then transferred to sterile polypropylene tubes and stored at –70 °C until extraction.

Contamination controls

All pre- and post-PCR manipulations on the samples analysed in this study were undertaken in physically separated rooms. The laminar flow cabinet where all pre-PCR sample manipulations were undertaken was decontaminated with undiluted industrial strength bleach prior to each use. All supplies and reagents used in this study were rendered DNA-free using methods suitable for the material being treated. All racks, pipettors, and containers were wiped with undiluted bleach prior to use. Pipette tips and tubes were autoclaved in small batches. Reagents were exposed to UV light for a minimum of 20 minutes to denature any decontaminating DNA (*e.g.*, Ou *et al.*, 1991); those reagents known to degrade when exposed to UV irradiation were autoclaved instead. Both extraction and negative (no template) PCR controls were used to detect the presence of systematic contamination. Positive control material was added to PCR reaction vessels only in the post-PCR area. To detect spurious contamination, all samples were extracted in duplicate and PCR amplifications from each extract were executed over multiple events.

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[3] 10 % v/v solution composed of industrial strength bleach containing 12% sodium hypochlorite.

Extraction

All samples were extracted using a guanidium thiocyanate protocol first proposed by Boom and others (1990) with several modifications. The modified protocol used 0.5 grams of bone per extract, incubated in extraction buffer overnight at 65 °C for a minimum of 16 hours. This was followed by a silica binding step where the bone supernatant is incubated with 500 µL of extraction buffer and 40 µL of silica and placed on a rotator at room temperature for two hours. The silica pellets were washed twice; first with wash buffer, then 70 % ethanol, followed by a single wash with acetone. After the silica pellets were dry, the DNA was eluted from the silica with 100 µL of water in a 56 °C water bath for one hour. The resulting DNA extracts were transferred to sterile microfuge tubes and stored at –20 °C until analysis.

PCR amplification

PCR amplifications were performed using either a Perkin-Elmer 2400 thermocycler (Foster City, CA) or a MJ TC Minicycler (MJ Research, Boston, MA). Each 50 µL reaction mix consisted of 5 µL 10X PCR Buffer (Invitrogen), 0.2mM of each dNTP (PE Biosystems), 1.5 mM $MgCl_2$ (Invitrogen), 200 pmol of each relevant primer, 15 µg of BSA (NEB), 1.25 U of Platinum *Taq* DNA Polymerase (Invitrogen). DNA extracts were not quantitated; instead, a standard 8 µL of template was added to each reaction mixture.

All primers used in this study and their respective annealing temperatures are listed in **Appendix 3** and represent primers derived from other aDNA studies in addition to those designed within this study. The primers created to flank the *CfoI* site loss at np 7598 were originally designed to identify haplogroup E individuals. However, recent modifications to the Asian mtDNA tree suggest that Siberian individuals harbouring the *CfoI* 7598 site loss actually belong to haplogroup G2a (Yao *et al.*, 2002) as haplogroup E has only been characterised in Tibetian (Torroni *et al.*, 1994) and southern Chinese groups (Kvisvild *et al.*, 2001) at negligible frequencies (Derenko *et al.*, 2003). Haplogroup G2a status has been assigned in this study based on *CfoI* status and representative HVI sequence variants. Amplifications using the PE 2400 thermocycler consisted of an initial denaturation step at 94 °C for 2 minutes, followed by 40 cycles of 30 s at 94 °C, 1 min at relevant annealing temperature and 30 s at 72 °C. Reaction conditions using the MJ PTC Minicycler were modified slightly, in that denaturation took place at 95 °C for 60s and the annealing and extension steps were increased to 90s and 60s respectively. Regardless of the thermocycler used, amplifications were completed with a final extension step of 5 minutes at 72 °C.

The limited amount of sample available for analysis in this study necessitated a low resolution RFLP approach in which samples were initially amplified using primers to detect the presence or absence of the *AluI* 10397 site gain characterising haplogroup M. Those individuals belonging to haplogroup M were further characterised using PCR primers flanking the RFLPs defining haplogroup C, D and G2a. Those individuals lacking the *AluI* 10397 site gain were further defined using primers flanking polymorphisms characteristic of haplogroups A, B, F and in certain cases, the *DdeI* 10394 site gain as well as the *Dde I* 1715 site loss characterising haplogroup X.

RFLP haplogroup assignment

Amplified PCR products were digested with restriction enzymes characterising the Asian haplogroups M, defined by an *AluI/DdeI* site gain at nucleotide pair (np) 10397/10394; A, by the *HaeIII* site gain at np 663; C by the *HincII* site loss at np 16359; D by an *AluI* site loss at np 5176; F by a *HincII* site loss at np12406, G2a by a *CfoI* 7598 site loss and X by the *DdeI* 1715 site loss. Restriction digests were prepared by adding 5 units of relevant restriction enzyme and 1 µL of 10X buffer (NEB or Invitrogen) diluted to a final volume of 10 µL with sterile water to the entire volume of amplified product. All restriction digests were incubated overnight at 37 °C. The digested products were visualised using a Fluor-S Multimager with the Quantity One software package (BioRad) after electrophoresis on 12 % polyacrylamide gels and ethidium bromide (10 mg/mL) staining.

Sequencing of a portion of the mtDNA HVI was performed using primers flanking a 176 bp region of HVI from positions 16191 to 16367; the sequences for these primers are also listed in Appendix 3. This fragment was targeted because it contains a majority of the informative polymorphisms characterising Asian HVI variation. The primary sequencing PCR reaction reagent concentrations and conditions were the same as those used for RFLP amplifications except that 50, rather than 40 cycles of PCR were performed. The primary PCR products for cycle sequencing were purified using the QuickStep 2 PCR Purification kit (Edge Biosystems). Sequencing of 75 ng of template was undertaken for both the H and L strands using an ABI 377 sequencer and the BigDye terminator package (Perkin Elmer) following standard manufacturer specifications. The resulting sequence data were read manually and deviations from the Cambridge reference sequence (Anderson *et al.*, 1981) were noted.

Evaluation of authenticity

Results from this study were only considered authentic if reproduced from a minimum of two independent extractions and multiple amplification events. HVI sequencing was used to screen samples for contamination derived from curation activities (see Chapter 2) and samples with sequences matching those of reference samples were eliminated from the analysis. Samples with HVI polymorphisms not typical of a given haplogroup motif were also considered to be non-authentic, as were sequences with non-reproducible heteroplasmy. Logistical and financial constraints prevented us from replicating all of the markers by independent investigators; however, HVI sequencing was performed by a different laboratory and thus serves to corroborate the authenticity of the RFLP data.

Statistical analysis

Modern Eurasian population data were incorporated into this study to provide a comparative framework whereby the contribution of the Kitoi and Serovo-Glazkovo to the subsequent population history of Siberia might be estimated. Only modern populations with comprehensive mtDNA RFLP haplogroup assignments were used in this study as the HVI dataset for Lokomotiv and Ust-Ida is to date, incomplete. **Figure 4-2** shows approximate geographic locations of these groups. The populations used for comparative analysis span most of Siberia and parts of Central and East Asia. Siberian groups from the Altai-Sayan plateau to the west of Lake Baikal include the Khakassians, Altai-Kizhi, South Altai, Shorians, Tofalars, Sojots, Tuvinians, Todjins (Derenko *et al.*, 2001; 2002a; 2003). The Buryats sampled by Derenko and colleagues (2002a; 2003) span the entire Buryat republic which is bounded by Lake Baikal on the west and Mongolia and China to the south. Groups inhabiting the Yenisei and

Ob river basins to the northwest of Cis-Baikal include the Kets and Mansi (Derbeneva *et al.*, 2002a; 2002b). The Evenks and the Yakut datasets compiled by Derenko and colleagues (2002a) were sampled from various communities to the north and east of Lake Baikal in the Evenk Autonomous okrug (Yakuts and Evenki) and the Sakha-Yakutia Republic (Yakuts). A large Siberian dataset assembled by Torroni and others (1993) which includes the Eskimos, Chukchi, Koryaks, Evens, Yukagirs, Nganasans, Selkups, Nivikhs, Evenks and Udegeys was also used. The augmented Chuckchi and Koryak datasets constructed by Derbeneva and colleagues (1998) and Schurr and colleagues (1999) respectively were also used for comparison. The Central and East Asian populations chosen include Tibetians, (Torroni *et al.*, 1994), Mongolians (Merriwether *et al.*, 1996) and Koreans (Snall *et al.*, 2002). The Mongolian mtDNA dataset was constructed from individuals living in the city of Ulanbataar, located in Northern Mongolia. The geographic locations of the Tibetian and Korean communities used in this study are not indicated on this map as they fall outside its margins. Haplogroup assignments were also derived from HVI data obtained from a northern Mongolian cemetery population known as Egyin Gol, dating to the 3rd century BC (Keyser-Tracqui *et al.*, 2003). Egyin Gol is located on the Selenga river which drains into Lake Baikal. Because of the relative geographic proximity of Egyin Gol to Lokomotiv and Ust-Ida, the inclusion of this cemetery in the analysis allows the population structure of prehistoric Siberia to be better characterised.

Figure 4-2: Map of modern Siberian populations used for comparison in this study

RUSSIA
MONGOLIA
www.lib.utexas.edu/maps

The mtDNA haplogroup distributions of prehistoric and modern populations were compared using a number of statistical algorithms. An exact test of population differentiation (*i.e.*, Raymond and Rousset, 1995) was used to test the null hypothesis that the groups at Lokomotiv and Ust-Ida represent a homogenous population using *Arlequin* 2.000 statistical package (Schneider et. al., 2000). This exact test is considered to be analogous to a Fisher's exact test with a two-by-two contingency table expanded to a table of a size defined by the number of populations by the number of haplogroups examined in this study. An exact test approach is preferable when dealing with small sample sizes as the chi-square test for homogeneity assumes that any given cell has a minimum frequency of five (Gould and Gould, 2002).

Biological distances between prehistoric and modern populations were estimated by R-matrix analysis (Harpending and Jenkins, 1973) using ANTANA (Harpending and Rogers, 1984). A plot of the first two principal components (PC) of the covariance matrix was constructed in MINITAB 13.3, allowing the biological distance between all populations to be observed in the form of a map. To assess the rigour of the R-matrix estimates, biological distances were also computed in *Arlequin* using a F_{ST} algorithm which also estimates the variance of mtDNA haplogroup distributions between pairs of populations. Computing F_{ST} estimates in *Arlequin* has an additional advantage in that P values are computed, allowing the statistical significance of the observed estimates to be assessed. The F_{ST} estimates were also treated by principal components analysis (PCA) in MINITAB and a plot representing the first two components was constructed.

To assess the relative mtDNA diversity and community size of the Kitoi and Serovo-Glazkovo, gene diversity (*h*; Nei and Roychoudhury, 1974; Nei, 1987) estimates for both the Neolithic and modern Asian groups were calculated in *Arlequin* (Schneider *et al.*, 2000). By plotting *h* against the deviations of the biological distances from the centroid of distribution (*i.e.*, *rii*) calculated in ANTANA (Harpending and Rogers, 1984) it may be possible to assess the relative amounts of gene flow or genetic drift acting on a population (*e.g.*, McComb *et al.*, 1996; Crawford *et al.*, 2002; Rubicz *et al.*, 2002). Genetically isolated populations are predicted to have larger r_{ii} values and decreased heterozygosity due to the effects of genetic drift; thus, such groups will be found below the theoretical regression line. In contrast, a population experiencing large amounts of gene flow will have smaller r_{ii} values and high heterozygosity values, placing them above the theoretical regression line.

Results and Discussion

Analytical success

Authentic mtDNA RFLP data were retrieved from 31 of 40 (78%) Lokomotiv and 39 of 42 (93%) Ust-Ida individuals. Preliminary mtDNA HVI sequence analysis has produced reproducible HVI variants concordant with RFLP assignments in 10 of 31 (32%) Lokomotiv and 17 of 39 (44 %) Ust-Ida individuals. The reduced retrieval rate of authentic RFLP and HVI data from Lokomotiv samples compared with those from Ust-Ida can be attributed to the increased contamination of skeletal material, believed to have occurred through osteological examination of the commingled communal burials (see Chapter 2). Equivocal HVI sequence data from Lokomotiv and Ust-Ida represented both contaminating sequences derived from modern DNA and ambiguous sequence variants, expected to stem from postmortem DNA modification that were not concordant with published haplogroup motifs.

Neolithic mtDNA haplogroup assignments

A combined strategy of RFLP analysis and HVI sequencing identified five characteristic Asian mtDNA haplogroups in 28 of 31 (90%) Lokomotiv and 30 of 39 (77 %) Ust-Ida individuals. The haplogroup frequencies for Lokomotiv, Ust-Ida and all other populations included for comparative analysis in this study are presented in **Appendix 4**. Although all five haplogroups are shared between individuals at Lokomotiv and Ust-Ida, the frequency distributions are remarkably different (**Table 4-1**). Haplogroup frequencies at Lokomotiv include four A (13 %), one C (3%), seven D (23%), one G2a (3%) and fifteen F (48%) individuals, while the haplogroup distribution at Ust-Ida consists of ten A (26%), eleven C (28%), two D (5%), four G2a (10%) and three F (8%) individuals.

Neither haplogroup B nor X were found in either Lokomotiv or Ust-Ida. A summarised dataset of all RFLP and HVI amplifications conducted for Lokomotiv and Ust-Ida can be found in **Appendix 5a** while examples of RFLP data produced for this experiment are shown in **Appendix 5b**.

Table 4-1: Distribution of mtDNA haplogroups in Neolithic Siberian cemetery populations

Group	n	A	C	D	F	G2a	Other
Lokomotiv	31	4 (13%)	1 (3%)	7 (23%)	15 (48%)	1 (3%)	3 (10%)
Ust-Ida	39	10 (26%)	11 (28%)	2 (5%)	3 (8%)	4 (10%)	9 (23%)

Sequencing of the mtDNA HVI was also used to further resolve haplogroup assignments of two individuals from Lokomotiv and nine from Ust Ida who lacked polymorphisms defining haplogroups A to G2a and thus were classified as haplogroup 'Other'. The summarised HVI sequence data from Lokomotiv and Ust-Ida is presented in **Table 4-2**. The raw sequence data for each unique HVI lineage defined for Lokomotiv and Ust-Ida individuals can be found in **Appendix 5c**.

The entire set of haplogroup 'Other' individuals from Lokomotiv lack the *DdeI/AluI* 10394/10397 site gains as do three from Ust-Ida. As such, these individuals are expected to have polymorphisms representative of haplogroup N types which encompass both European and Asian mtDNA variants (*e.g.*, Kvisvild *et al.*, 2002). Both haplogroup N individuals from Lokomotiv and one from Ust-Ida possess HVI sequence variants characteristic of haplogroup U5a (16256 16270), predicted to have Eurasian origins with an estimated coalescence time of 50 000 years (Sykes, 1999).

Two other Ust-Ida haplogroup N individuals are characterised by a single T to C transition at 16311, a polymorphism found in both European and Asian populations, either alone as seen in haplogroup H, or in association with other

Table 4-2: HVI sequences found in the Kitoi and Serovo-Glazkovo

Hap	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Lokomotiv	Ust-
	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	(n = 10)	Ida
	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3		(n=17)
	2	2	3	4	5	6	7	7	8	9	9	0	1	1	2		
	3	7	2	9	6	2	0	8	8	0	8	4	1	9	7		
	C	A	C	T	C	C	C	C	T	C	T	T	T	G	C		
CRS	C	A	C	T	C	C	C	C	T	C	T	T	T	G	C		
A	T	.	.	.	.	.	.	.	.	T	.	.	.	A	.	2	3
A	T	G								T				A		0	2
C	T	.	.	.	.	.	.	.	.	.	C	.	.	.	T	1	2
	T										C						1
D	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.		2
	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2	
F	.	.	A	C	.	.	.	.	.	.	.	C	C	.	.	2	
G2a	T	G	.	.	.	.	.	T	.	.	.	.	.	.	.	1	
	T	G	.	.	.	T	.	T	.	.	.	.	.	.	.		1
U5a	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	2	1
Other	T	G				T		T					C				2
	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.		2
	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1

sequence variants as is the case with haplogroups within haplogroup N including A, D5, K, R, U4, U5a andU5b (Kvisvild *et al*., 2002; Kong *et al*., 2003). The presence of the 16311 variant in the absence of any other substitutions within the region sequenced effectively rules out all but haplogroup H, R, or U4; the latter two which have additional sequence variants falling outside the region sequenced in this study.

The remaining four haplogroup 'Other' individuals from Ust-Ida are defined by the *AluI* 10397 site gain characteristic of haplogroup M. Two Ust-Ida individuals have an HVI motif with substitutions at 16223 16227 16262 and 16278. While this lineage is identical to the G2a lineage characterised for one Ust-Ida individual, both of these other individuals lack the diagnostic G2a *CfoI* 7598 site loss. The remaining Ust-Ida haplogroup M individual for whom an HVI motif has been defined has a sole C to T transition at 16223; when seen alone, this substitution may correspond to either haplogroup G4 or an undifferentiated haplogroup M lineage (Kivisvild *et al.*, 2002; Yao *et al.*, 2003). The HVI status of the three remaining haplogroup 'Other' individuals awaits confirmatory sequencing. By evaluating these haplogroup 'Other' individuals for additional RFLP markers and sequencing additional fragments of HVI and HVII, their haplogroup status may be further resolved.

Biological distance of Kitoi and Serovo-Glazkovo

The principal intent of this study is to test the hypothesis that a depopulation event occurred in Cis-Baikal during the Middle Neolithic, causing an approximate 800-year hiatus in the population history of the region. This biological discontinuity hypothesis evolved with the discovery that no cemetery sites nesting the use of Lokomotiv and Ust-Ida have been found in the Cis-Baikal region (Weber, 1995; Weber *et al.*, 2002). By estimating the biological distance between the Kitoi and Serovo-Glazkovo through the examination of the differences in their respective mtDNA haplogroup distributions, an additional line of evidence to test the discontinuity hypothesis is obtained.

Although the Kitoi and Serovo-Glazkovo share all six mtDNA haplogroups identified in this study, their distributions are significantly different (P=0.000). These differences are seen largely in the moderate proportions of haplogroups A, C and G2a (at frequencies of 26, 28 and 10 % respectively) in Ust-Ida individuals; while the Lokomotiv sample is composed mostly of haplogroup D (23 %) and F (49 %). Thus, it seems likely that the Kitoi and Serovo-Glazkovo do not share common matrilineal population origins. Coupled with the gap in the archaeological record, together with differences in mortuary ritual and subsistence strategies, the disparate mtDNA haplogroup distributions between the Kitoi and Serovo-Glazkovo strongly suggest that a population shift occurred after a biological hiatus in the Cis-Baikal region during the seventh millennium BP.

However, explaining the mechanisms by which two biologically distinct groups emerged in the Cis-Baikal Neolithic is a complex task that is beyond the scope of this dissertation. Both cultural and environmental factors have to be taken into consideration which is a subject of research by other modules within the Baikal Archaeology Project (BAP). Archaeological evidence suggests that the Kitoi social relations were shaped by power and sex imbalances (Weber *et al.*, 2002); if such social complexities created intra-community tension, this may have precipitated the dispersal of the group. Alternatively, if the climate in the Cis-Baikal became inhospitable during the Middle Neolithic or if resources became scarce, the Kitoi may have left the region to settle elsewhere. In both of these cases, the Serovo-Glazkovo may then represent a different population who migrated into the area. If the relocation of the Kitoi and subsequent migration of the Serovo-Glazkovo caused the population shift observed in Cis-Baikal, we might see population affinities and ethnographic similarities between the Kitoi and groups in other regions of Siberia. This approach will be explored below with the statistical estimation of prehistoric and modern Asian population affinities.

Instead of the migration hypotheses, it may be that the Kitoi were afflicted by population stresses that eventually contributed to their demise (Link, 1996; Link, 1999; Weber *et al.*, 2002). The Kitoi are believed to have had lower reproduction rates than the Serovo-Glazkovo based largely on the lower frequency of subadults in the mortuary record at Lokomotiv compared with Ust-Ida. A greater degree of enamel hypoplasia (*i.e.*, horizontal striations in tooth enamel) was observed in the Kitoi compared with the Serovo-Glazkovo. As enamel hypoplasia is thought to be a marker of nutritional stress during childhood, its presence suggests that Kitoi food procurement may have been inconsistent.

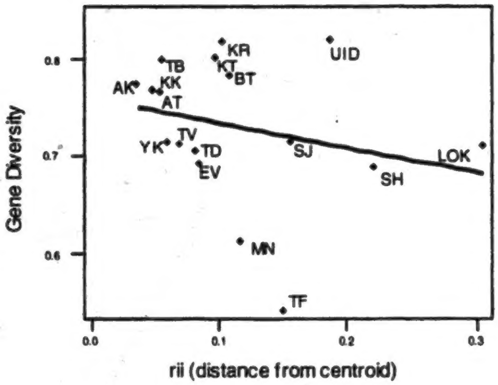
The Kitoi also appeared to have a lower life expectancy than the Serovo-Glazkovo as suggested by the increased frequency of individuals in the 35 to 50 year age class buried at Lokomotiv compared to Ust-Ida whose largest age class consisted of individuals greater than 50 years old (Link, 1996). However, other than osteoarthritis which is found in both cemeteries, there is little osteological evidence to suggest that either the Kitoi or Serovo-Glazkovo were affected by disease (Lieverse, 1999; Weber *et al.*, 2002). By examining subsistence strategies and mobility patterns as well as the distribution and size of cemetery sites throughout the Cis-Baikal region, Weber and colleagues (2002) proposed that while at least a few of the Kitoi communities (*i.e.*, Lokomotiv and Kitoi on the Angara river as well as Shamanka II on Lake Baikal) were likely to have been larger than those of the Serovo-Glazkovo, they were socially and reproductively isolated units. Unlike the Serovo-Glazkovo, whose high mobility would have promoted population growth through the actions of migration and gene flow, the combined effects of genetic isolation and diminished health and fertility in the Kitoi may have resulted in their demise.

While the mtDNA variation catalogued in this study reveals nothing about the health of the Kitoi, it is possible to test Weber and colleagues (2002) suggestion that the genetic isolation of the Kitoi contributed to their disappearance from the Cis-Baikal region. **Figure 4-3** shows a plot of *h* (Nei and Roychoudhury, 1974; Nei, 1987) versus r_{ii} for the Kitoi (Lok), Serovo-Glazkovo (UID) and modern Asian groups used for comparison in this study. Groups such as the modern Siberian Tofalars (TF) who are relatively small and genetically isolated (Derenko *et al.*, 2003), are positioned below the theoretical regression line and away from the centroid of distribution. Large, highly diverse Siberian groups such as the Altai (AT), Kets (KT) and Buryats (BT) are positioned above the regression line.

The position of UID above the regression line corroborates Weber and colleagues (2002) notion that the mobility patterns of the Serovo-Glazkovo promoted gene flow through interaction with other regional groups (*e.g.*, McComb *et al.*, 1996; Crawford *et al.*, 2002). Similarly, the moderate mtDNA diversity observed for the LOK combined with their large r_{ii} is consistent with the idea that LOK formed a relatively large but genetically isolated Kitoi community.

Although somewhat misleading in this context given the eight millennia separating the Kitoi from modern Siberians, the apparent biological distance separating the Kitoi from most other Siberian groups may provide clues to their fate and will be discussed in detail below.

Figure 4.3: Plot of mtDNA diversity against biological distance for prehistoric and modern Asian groups. LOK = Lokomotiv, UID=Ust-Ida, AK=Altai-Kizkhi, AT=Altai, BT=Buryats, EV=Evenki, KT=Kets, KR=Koreans, KK=Khakassians, MN=Mansi, SH=Shorians, SJ=Sojots, TB=Tibetians, TD=Todjins, TF=Tofalars, TV=Tuvinians, YK=Yakuts



If the Kitoi were diminishing in size over time, an increased amount of mtDNA homogeneity among individuals (*i.e.*, a genetic bottleneck) should be observed towards the latter stages of cemetery use at Lokomotiv. **Table 4-3** shows the distribution of Kitoi mtDNA haplogroups across three arbitrary chronological periods. When these distributions are compared using an exact test in *Arlequin*, they are not observed to significantly differ suggesting that the matrilineal population structure of the Kitoi remained relatively stable throughout the time Lokomotiv was used. Thus, it seems unlikely that the cessation of Lokomotiv use coincided with the extinction of the Kitoi. The impending aDNA analysis of the other major Kitoi cemetery in Cis-Baikal, Shamanka II (Bazaliiski *et al*., 2003) will provide further insight into the fate of the Kitoi. However, if population affinities are not observed to be shared between the Kitoi and other modern Siberian groups, it may be that the Kitoi did not contribute to the subsequent population structure of Siberia.

Table 4-3: Chronological distribution of Kitoi mtDNA haplogroups

mtDNA Haplogroup	7140–6750BP	6740–6620 BP	6620–6200 BP
A	1	2	1
C	0	1	0
D	2	2	3
F	5	6	4
G2a	1	0	0
U5a	0	0	2
Other	1	0	0
Total	10	11	10

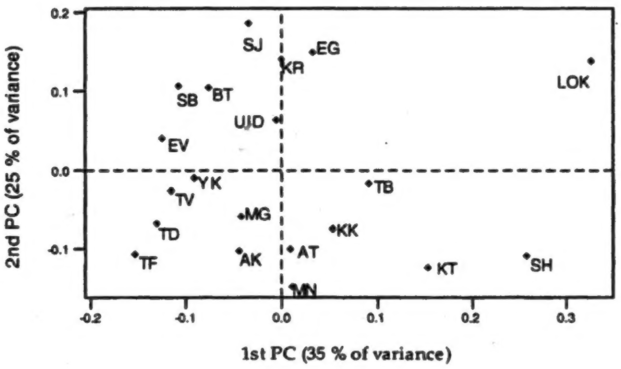
Population affinities of Kitoi and Serovo-Glazkovo

Population genetic models reflect the assumption that populations with similar mtDNA haplogroup distributions are more likely to share a common maternal ancestry than those groups whose distributions are disparate (*e.g.*, Kaestle and Horsburgh, 2002). Through analysis of molecular variance (*i.e.*, AMOVA) testing of mtDNA RFLP and HVI data, Derenko and colleagues (2003) have suggested that modern Siberian populations do not cluster by language or anthropological (*i.e.*, phenotypic) variation; instead, only geography was seen to significantly influence biological distance between groups. Consequently, shared matrilineal population affinities between the Kitoi and modern Cis-Baikal groups might suggest that matrilineal ancestors of the Kitoi remained in the Cis-Baikal region. If the Kitoi are instead observed to share similar mtDNA haplogroup distributions with modern groups outside the Cis-Baikal region, this may indicate that the Kitoi left Cis-Baikal and settled elsewhere. Likewise, it may be possible to demonstrate population continuity in the Cis-Baikal region from the Neolithic through to modern times if biological distances between the Serovo-Glazkovo and modern Cis-Baikal populations are small. To answer these questions, biological distances were estimated between prehistoric and modern Asian populations; the subsequent patterning was then evaluated to seek meaning from the obtained biological distances.

Kitoi population affinities

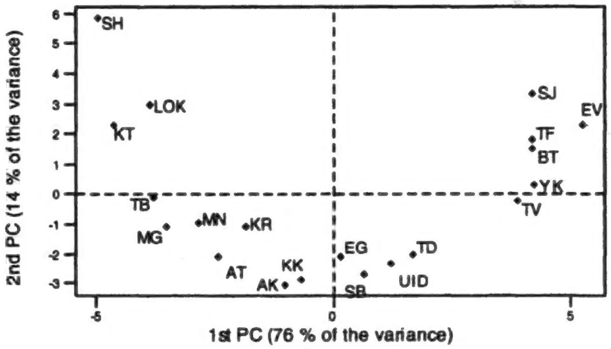
The PC map constructed from the first two principal components of the R-matrix representing mtDNA haplogroup distributions in prehistoric and modern Asian populations can be seen in **Figure 4-4**. The first two components explain 60 % of the total variance arising from the mtDNA haplogroup distributions. At first glance, most modern populations appear to cluster as a function of physical distance, thus corroborating the observation of Derenko and colleagues (2003). Two general clusters are seen; the cluster occupying the bottom left portion of the PC map is comprised of modern groups inhabiting the Ob and Yenisei river basins to the west and north of Cis-Baikal while the other cluster is composed of groups living east of the Yenisei and those living to the northeast of Cis-Baikal. The Tibetans and Koreans occupy a central position on the map, attesting to their moderate frequencies of the CfoI 7598 site loss characterising haplogroup E and G2a (e.g., Torroni *et al.*, 1994; Snall *et al.*, 2001). Interestingly, Mongolians (Merriwether *et al.*, 1996) group with the Yenisei and Ob basin groups, although one might expect them to be more proximate to Buryats and Sojots (e.g., Derenko *et al.*, 2002a). While the Altai Mountains are geographically contiguous with Mongolia and both these regions demonstrate shared language and cultural affiliations (e.g., Crawford *et al.*, 2002), the association may be artifactual. The Mongolian RFLP dataset has only been completely characterised for haplogroups A to D; as such, it is seemingly the large component of 'Other' haplogroups in the Mongolian sample creating this association with the Altaic groups.

Figure 4-4: PCA of R-matrix for prehistoric and modern Asian mtDNA Data Key for population groups: LOK = Lokomotiv, UID=Ust-Ida, AK=Altai-Kizkhi, AT=Altai, BT=Buryats, EG=Egyin Gol, EV=Evenki, KT=Kets, KR=Koreans, KK=Khakassians, MN=Mansi, SH=Shorians, SJ=Sojots, TB=Tibetians, TD=Todjins, TF=Tofalars, TV=Tuvinians, YK=Yakuts



While modern Siberian populations are seen to cluster according to geographic distribution, the prehistoric samples examined in this study do not fit this pattern. The Kitoi, with 72 % of the total haplogroup distribution attributed to the frequencies of haplogroup F (49 %) and D (22 %) do not cluster with modern groups such as the Sojots, Buryats and Tuvinians who occupy regions close to Lake Baikal. Instead, it sits in an isolated position on the PC map with greater proximity to the groups living in the Yenisei and Ob river basins such as the Shorians and Kets. The affinity of the Kitoi with the Shorians and Kets is confirmed by the clustering of the three populations in the F_{ST} PC plot shown in **Figure 4-5**. Interestingly, the biological distances between the Kitoi and Shorians as well as the Kitoi and Kets estimated by F_{ST} are not statistically significant with respective P values of 0.21 and 0.06 (data not shown).

Figure 4-5: F_{ST} PC plot of modern and prehistoric Asian groups. Key for population groups: LOK=Lokomotiv, UID=Ust-Ida, SH=Shorians, KT=Kets, TB=Tibetans, KK=Khakkassians, AT=Altai, AK=Altai-Kizhi, MG=Mongolians, TF=Tofalars, TD= Todjins, TV=Tuvinians, YK=Yakuts, EV=Evenki, SB=Siberians, BT=Buryats, SJ=Sojots, KR=Koreans, EG=Egyin Gol.



Shorians have a combined haplogroup F and D frequency of 50 % (Derenko *et al.*, 2001a; 2002a); haplogroup D, with an occurrence of 10 % is the second largest Asian component in this group. Shorians also have the highest frequencies of European mtDNA types (36%) seen in Western Siberian populations, of which the most prevalent haplogroup is H. Potapov (1964) suggested that phenotypically, Shorians resemble Uralic (Samoyedic) groups including the Kets and Mansi more than other Altaic groups. The Shorians share ethnographic affinities with the Kets who inhabit the Middle to Lower Yenisey river basin and speak a unique language (*i.e.*, Ket) that is neither Samoyedic nor Turkic in origin (Popov and Dolgikh, 1964). The Kets have the second highest frequency of haplogroup F (24%) seen in Siberian populations but have low frequencies of D (3%; Derbeneva *et al.*, 2002). However, Kets are also similar to Lokomotiv individuals in that they have moderate proportions of haplogroup A (8%) and lack haplogroup B.

When similarities between HVI data are sought, we see that the modern Kets and the Neolithic Kitoi share a single U5a lineage (16256 16270) at frequencies of five and ten % respectively. Similarly, common haplogroup A (16223 16290 16319) C (16223 16298 16327) and F (16232 16249 16304 6311) lineages are also seen in these two groups. Therefore, Kets share a minimum of 50 % of their HVI lineages with the Kitoi. Further characterisation of Lokomotiv HVI polymorphisms might increase this proportion. Unfortunately, an analogous comparison between the Kitoi and Shorians can not be made as a Shorian HVI dataset has not yet been published.

Although the similar matrilineal structure of the prehistoric Kitoi and modern Shorians and Kets speaks to population affinities between these three groups, the limited osteological evidence known for the Kitoi is not concordant with such a scenario. Gerasimova (1991; cited in Weber, 1995) believed Kitoi crania to have overt Mongoloid characteristics based on a sample analysed from the Selenga river basin cemetery known as Fofanovo. Such a phenotype contrasts with the more Europoid features defining the Uralic type which defines both the Shorians and the Kets (Potapov, 1964; Popov and Dolgikh, 1964). A comprehensive examination of Siberian phenotypic variation is beyond the scope of this thesis. However, given that the anthropological type defining the Kitoi has been proposed from the analysis of just one cemetery sample, a comprehensive examination of Kitoi cranial phenotypic traits from both Lokomotiv and Shamanka II will be valuable to gain further insight into Kitoi population affinities.

Interestingly, there are a few notable geographic and ethnographic affinities between the Kets and the Kitoi. The Kets and the Kitoi are connected by the Angara/Yenisei watershed which could easily serve as a natural migratory route for groups living on the upper Angara river. The first Russians to reach the Yenisei noted the presence of a Ket group known as the Asans on the lower reaches of the Angara river near the confluence of the Yenisei, although the Asans were later thought to merge with Evenks who today occupy territory immediately to the east of where modern Kets live (Popov and Dolgikh, 1964). While many Kets are hunters, there is a strong fishing tradition among northern Kets, just as is seen in the Kitoi. Popov and Dolgikh (1964) remark on the sacrifice and burial of dogs with the dead as a Kettic mortuary tradition. A similar ritual was practiced by the Kitoi as a burial at Lokomotiv was discovered containing the remains of a wolf in association with a human skull (Bazaliiskiy and Savelyev, 2003).

In contrast, no such affinities are observed between the Shorians and the Kitoi. The Shorians use hunting as a primary subsistence strategy and only recently began burying their dead in the ground; before the 20th century, the Shorians wrapped their dead in birch bark and deposited them in logs (Potapov, 1964). Therefore, although the Kitoi, Kets and Shorians share similar mtDNA haplogroup distributions, it seems that overall, there are stronger affinities between the Neolithic Kitoi and modern Kets.

Given that large biological distances exist between the Kitoi and modern Siberian populations who live proximate to Cis-Baikal, it is reasonable to consider that the Kitoi left the upper reaches of the Angara river to settle along the Yenisey river basin. The interaction of the Kitoi with regional groups along the Yenisey may have resulted in the creation of a population from which the founders of the Kets, Shorians and other similar groups evolved. In the future, it would be desirable to corroborate this hypothesis with Y-chromosome data, the analysis of which is underway (Schurr *et al.*, in progress). The Kitoi mtDNA dataset will also be enhanced with the analysis of mtDNA and Y-chromosome polymorphisms from Shamanka II which is currently being excavated (Bazaliiski, 2003). Similarly, the discovery of additional cemetery populations in the Angara and Yenisey basins may further illuminate the prehistoric population structure of Siberia.

Serovo-Glazkovo population affinities

The population affinities of the Serovo-Glazkovo are strikingly different than those of the Kitoi. The PC plot of the R-matrix analysis (**Figure 4-4**) sees the Serovo-Glazkovo fall into the milieu of modern Siberian populations who live east of the Yenisei and those proximate to Cis-Baikal. This association between these groups appears to be due largely to the similar distributions of haplogroup C and F observed. Where the Serovo-Glazkovo and these modern groups differ is in their dissimilar frequencies of haplogroups D and G2a. While the Serovo-Glazkovo have a small frequency of haplogroup D (5%) and a moderate frequency of G2a (10%), the opposite is observed for most other Siberian groups living between the Yenisei and Lake Baikal. The only exceptions are the Sojots and Buryats who have moderate frequencies of haplogroup G2a (9 and 14% respectively) in association with high frequencies of haplogroup D (50 and 33 %). Intriguingly, the Serovo-Glazkovo also harbour haplogroup A at a frequency of 26 %, higher than almost all other Asian populations discussed in this study. The only other Siberian populations known to have comparable frequencies of haplogroup A are the Chukchi (38%) and Koryaks (24%) sampled by Torroni and colleagues (1993), who inhabit Northeastern Siberia. In contrast, the average haplogroup A frequency of modern Siberian populations living more proximate to Lake Baikal is around 4 % (Derenko *et al.*, 2002a).

The PC plot of the F_{ST} estimates (**Figure 4-5**) reveals an association between the Serovo-Glazkovo, Todjins (Derenko *et al.*, 2002a), the large Siberian group sampled by Torroni and colleagues (1993) and the group from Egyin Gol (Keyser-Tracqui *et al.*, 2003). The F_{ST} P values associated with this cluster suggest that significant biological distances do not exist between the Serovo-Glazkovo and each of these groups (data not shown). The Serovo-Glazkovo and Todjins share similar distributions of haplogroups C and D and F; however, it seems unlikely that the Serovo-Glazkovo contributed extensively to the formation of the Todjins as they have completely disparate distributions of haplogroups A and G2a. While the Serovo-Glazkovo have moderate frequencies of both haplogroups, the Todjins have a low frequency of A (4%) and are completely devoid of G2a. Instead of G2a, the Todjins possess moderate frequencies (19%) of haplogroup G4 (Derenko *et al.*, 2003) which has the HaeII site gain at np 4830 shared with G2a, but lacks the G2a CfoI 7598 site loss. In addition, the different HVI motifs of G4 (16223 16260 16325 16362) and G2a (16223 16227 16278 16362) suggest that these lineages diverged some time ago; indeed, Derenko and colleagues (2003) estimate the coalescence time of haplogroup G2 to be 27 000 ± 12 400 BP.

When similarities are sought between the limited mtDNA HVI dataset of the Serovo-Glazkovo and that of the Todjins (Derenko *et al.*, 2003), several lineages are seen to be shared between the two groups. The haplogroup A motif of 16223 16290 16319 defining more than half of the Serovo-Glazkovo HVI lineages is the sole haplogroup A motif characterised for the Todjins. Furthermore, the only haplogroup C lineage defined thus far for the Serovo-Glazkovo (*i.e.*, 16223 16298 16327) is one of the two majority C lineages found in the Todjins. Finally, the Serovo-Glazkovo haplogroup 'Other' motif characterised by the sole 16311 C-T transition may correspond to the haplogroup R motif of 16051 16168 16172 16311 seen exclusively in the Todjins. While it appears likely that some population affinity exists between these Serovo-Glazkovo and the Todjins, the extent of their relationship may never be known given the complex series of migrations that have defined the population history of this region over the last several millennia (*e.g.*, Levin and Potapov; 1964; Derenko, 2003, Pakendorf *et al.*, 2003). However, future expansion of the Serovo-Glazkovo RFLP and HVI datasets may highlight additional affinities with the Todjins that are not currently apparent.

The similar distributions of haplogroups A, C and D together with the absence of haplogroup B in the Serovo-Glazkovo and the circumarctic Chukchi and Koryaks (Torroni *et al.*, 1993) suggests that population affinities may be shared between these groups. However, subsequent sampling of the Koryaks by Schurr and others (1999) identifying haplogroups G4, Y and Z weakens this association as none of these variants are present in the Serovo-Glazkovo. However, haplogroup Y and Z have recently been reported in the aforementioned groups from the Tuva Republic (Derenko, 2003), suggesting these groups may share population affinities stronger affinities with the Koryaks. Subsequently, a reanalysis of F_{ST} estimates using enhanced Chukchi (Torroni *et al*; Starikovskya *et al.*, 1998) and Koryak (Torroni *et al.*, 1993; Schurr *et al.*, 1999) datasets suggest that the Serovo-Glazkovo did not likely make any identifiable contribution to the population structure of either the Chukchi (P=0.00) or Koryaks (P=0.01).

An ancient link between the Serovo-Glazkovo and the Huns?

There is also an observed association between Serovo-Glazkovo and the cemetery population from Egyin Gol (Keyser-Tracqui *et al.*, 2003), located in the Selenga river basin that drains into Lake Baikal on its west side. Egyin Gol was used for approximately five centuries by a group known as the Xiongnu or Huns (Keyser-Tracqui *et al.*, 2003). The Huns were nomadic pastoralists who inhabited and controlled a large part of Mongolia and Trans-Baikal from the third century B.C. to the third century A.D. at which time their realm of influence was diminished by the emergence of the Chinese Han dynasty (Marx, 2000). Few inferences about the mortuary behaviour of the Huns can be made from Keyser-Tracqui and colleagues (2003) study except for the notable patterning of a single affluent burial surrounded by double interments. Such patterning is also reported to have been practiced by the Sakha (*i.e.*, Yakut), a group inhabiting the Lena River basin north of Lake Baikal (Francfort *et al.*, 2000; cited in Keyser-Tracqui, 2003). Similar mortuary ritual has not been observed at Ust-Ida which is not surprising given that the Serovo-Glazkovo are thought to have a relatively egalitarian social structure (Weber, 1995; Weber *et al.*, 2002).

However, both these cemetery populations share the same Asian mtDNA haplogroups with the exception of one haplogroup B individual excavated from Egyin Gol. Although the Egyin Gol group has a higher proportion of haplogroup D (37%) and a lower frequency of G2a (2%) than the Serovo-Glazkovo who have D and G2a frequencies of 5 and 10% respectively, the proportions of haplogroups A, C and F are similar. Many HVI motifs are also shared between the Serovo-Glazkovo and those at Egyin Gol. The single haplogroup A motif found in the Serovo-Glazkovo is also found in 75 % of Egyin Gol sample. Likewise, 66% of the C, 50% of the F and 100% of the U5a HVI variants are shared between the two cemetery groups. However, the G2a motif in the Egyin Gol sample (16223 16227 16278 16362) varies from both G2a lineages found in the Serovo-Glazkovo (16223 16227 16262 16278). Interestingly, the G2a lineage characterising the Serovo-Glazkovo also vary from the single Kitoi G2a lineage (16223 16227 16278) and have not yet been characterised in any other modern Siberian group.

Russian scholars generally believe that the Xiongnu who occupied the Selenga river basin were immigrants who did not interact with the indigenous groups in the region (Okladnikov, 1964). However, the similarity of Serovo-Glazkovo and Xiongnu mtDNA haplogroup distributions suggest that gene flow may have occurred between descendents of the Serovo-Glazkovo and groups representing the northern reaches of the Huns. This association is compelling as it suggests that temporal stability was maintained in the regional matrilineal population structure of Lake Baikal for over four millennia (*i.e.*, from 4200 B.C. to 200 A.D.). Furthermore, the insignificant biological distance between the Serovo-Glazkovo and Xiongnu also reinforces the notion of a population shift in the Cis-Baikal region during the fifth millennia B.C. The Kitoi, who are biologically distinct from the Serovo-Glazkovo, also do not share obvious population affinities with the Xiongnu, making it even more unlikely that the Kitoi contributed to the subsequent population structure of the Cis-Baikal region.

Estimates of biological distance through R-matrix and F_{ST} analysis do not speak to strong population affinities between the Serovo-Glazkovo and modern Cis-Baikal groups. However, the relationship of the Serovo-Glazkovo with modern Evenk, Yakut and Buryat peoples should be explored as the oral histories of these groups pinpoint Lake Baikal as their ancestral homeland (Lopatin, 1940; Okladnikov, 1964;Vasilevich and Smolyak, 1964; Pakendorf *et al.*, 2003). While the Yakuts and Evenks are observed to share common population affinities when measures of biological distance are applied, Buryats have significantly different mtDNA haplogroup distributions from either of these groups (*i.e.*, Derenko *et al.*, 2002a; Pakendorf *et al.*, 2003). Therefore, it is not apparent whether the Buryats and Yakuts share matrilineal population affinities (Pakendorf *et al.*, 2003). Likewise, the haplogroup distributions of the Serovo-Glazkovo compared with the Yakuts, Evenks and Buryats are disparate; however, just as is seen between the Serovo-Glazkovo and the Xiongnu, all Asian haplogroups other than B are observed in all these populations.

The haplogroup A motif characterised by substitutions at 16223 16290 16319 which was previously noted to be shared by the Serovo-Glazkovo and the Todjins also represents half of all A lineages found in the Yakuts and Buryats (Pakendorf *et al.*, 2003). However, the frequency of haplogroup A is much higher in the Serovo-Glazkovo than in any of these modern groups. While the Buryats and Yakuts are defined by a slightly different G2a lineage (*i.e.*, 16223 16227 16278) than that found in the Serovo-Glazkovo (*i.e.*, 16223 16227 16262 16278), the frequency of CfoI 7598 site loss defining this haplogroup is similar between the Buryats and Serovo-Glazkovo (14 and 10 % respectively; Derenko *et al.*, 2001a; 2003). Unfortunately, a similar comparison of HVI data between the Serovo-Glazkovo and the Evenks is not possible at this time as data characterising the HVI lineages in the Evenks has yet to be published.

While this study demonstrates the likelihood that a relatively stable matrilineal population structure was maintained in the Lake Baikal region from the time of the Serovo-Glazkovo through to the Xiongnu, the degree to which the Serovo-Glazkovo contributed to the population structure of modern Siberian groups is not clear. Further characterisation of both mtDNA and Y-chromosome lineages in the Serovo-Glazkovo from Ust-Ida as well as other Serovo-Glazkovo cemeteries known to exist in Cis-Baikal will further illuminate the biological structure of this group and its relationship to modern Siberians. To better understand the complexities obscuring the population origins of many modern Siberian groups, it may be a worthy endeavour to sample additional cemeteries from different archaeological periods throughout Central and East Asia. Likewise, the continued sampling of Siberian and East Asian populations for both mtDNA and Y-chromosome analysis will be necessary to help link the past with the present.

A principal tenet of Siberian population history holds that the western regions of Siberia were simultaneously inhabited by both Asian and Europoid groups since as early as the Palaeolithic (*i.e.*, Okladnikov, 1964). This region has long been seen as a junction between east and west and recent mtDNA evidence has reinforced this notion with the characterisation of many European mtDNA lineages in western Siberian groups (*e.g.*, Derenko *et al.*, 2001a; 2001b; 2002a; 2003). Whether this heterogeneity extended as far east as Lake Baikal is not yet clear. Okladnikov (1964) proposed that a group of Eastern Europeans inhabited the Lake Baikal region during the Upper Palaeolithic as evidenced by the similarity in material culture observed between the Upper Palaeolithic sites of Malta and Bu'ret with those of Eastern Europe. However, Okladnikov predicted that growth of Asian-specific groups in the region eventually resulted in the replacement of these Upper Palaeolithic Europeans. Therefore, modern populations in the region of Lake Baikal who have not exchanged genes with Russian groups are expected to have a higher proportion of Asian mtDNA polymorphisms than their western neighbours (e.g, Derenko *et al.*, 2001a; 2002a; 2003). The mtDNA lineages characterising the two Neolithic cemetery populations examined in this study generally support this assessment. Although the mtDNA datasets of neither the Kitoi nor the Serovo-Glazkovo are yet completely characterised, our limited RFLP and HVI datasets suggest that 90% of the Kitoi and 85% of the Serovo-Glazkovo mtDNA lineages are Asian in origin. Comparatively, the proportions of Asian haplogroups found in modern Siberian populations range from a minimum of 60% in the Altaians to a maximum of 92% in the Evenks. The only definitive non-Asian haplogroup identified in either population is haplogroup U5a. The geographic origins of haplogroup U5a are ambiguous, having been observed in both European and Asian populations (Derenko *et al.*, 2002b) but as previously noted, this haplogroup has great temporal depth. Haplogroup U5a has been detected both in many modern Siberian groups (*e.g.*, Derbeneva, 2002a; 2002b Derenko *et al.*, 2003; Pakendorf *et al.*, 2003) as well as other prehistoric Asian cemetery populations (e.g, Keyser-Tracqui *et al.*, 2003; Oota *et al.*, 1999). By exploring the geographic origins of haplogroup U5a, it may be possible to reveal another facet of Siberian population history.

Conclusions

This study reports the successful retrieval of authentic mtDNA haplogroup data from 70 individuals whose postmortem intervals range from 7200 to 4200 years B.P. To the best of our knowledge, this is the the largest and oldest skeletal sample for which aDNA data has ever been reported. Both mtDNA RFLP and HVI polymorphisms were examined from skeletal remains excavated from the Neolithic Lokomotiv and Ust-Ida cemeteries located on the Angara river basin immediately west of Lake Baikal. The principal objective of this study was to use mtDNA data to test the hypothesis that an 800-year biological and cultural hiatus occurred in the Cis-Baikal region during the 7th millennium BP. Similarly, by comparing the Neolithic mtDNA datasets with those published for modern Siberian populations, we sought to identify whether biological continuity could be demonstrated in the Cis-Baikal region from the Neolithic through to modern day.

Authentic mtDNA RFLP data were retrieved from 78% of Lokomotiv and 93% of Ust-Ida individuals. Sequencing of the mtDNA HVI is currently in progress; motifs corroborating the observed RFLP data have to date been retrieved from just over 40 % of both Lokomotiv and Ust-Ida series. Six mtDNA haplogroups were definitively identified in both the Kitoi, who used Lokomotiv, and the Serovo-Glazkovo, who buried their dead at Ust-Ida. Five of these haplogroups were of Asian origin having RFLP and HVI polymorphisms characteristic of haplogroups A, C, D, F, and G2a. The sixth haplogroup was identified solely from a mtDNA HVI motif characterising haplogroup U5a (16256 16270); haplogroup U5a has a considerable time depth and is thought to have originated in Western Eurasia (Sykes, 1999). The Serovo-Glazkovo also have a proportion of "Other" haplogroups that have not been fully identified at this time; however, limited RFLP and HVI characterisation place these individuals on both the M and N branches of the Asian mtDNA tree (*e.g.*, Kvisvild *et al.*, 2001).

Although both the Kitoi and Serovo-Glazkovo share most mtDNA haplogroups, the distributions of these haplogroups are significantly different. While the Kitoi harbour high frequencies of haplogroup F and moderate frequencies of haplogroup D, the Serovo-Glazkovo are defined by moderate frequencies of haplogroup A, C and G2a. The haplogroup distribution of the Kitoi places this group well outside the mtDNA population structure observed in groups inhabiting South-Central Siberia today. Interestingly, the Kitoi instead appear to share population affinities with two Yenisei river basin groups (*i.e.*, the Shorians and Kets). The disparate haplogroup distributions of the Kitoi and Serovo-Glazkovo combined with the observed 800-year gap in the archaeological record suggest that the Kitoi migrated out of the region and may have settled along the Yenisei where they contributed to the formation of groups inhabiting the region today.

Although the Serovo-Glazkovo are generally seen to cluster with modern Siberian populations living east of the Yenisei river when measures of biological distance are applied, obvious population affinities are not observed between the Serovo-Glazkovo and any modern Siberian group. The contribution of the Serovo-Glazkovo to the population structure of modern-day Siberia is likely to be obscured by a complex series of migration and admixture events that have shaped the character of many modern groups occupying the southern part of East Siberia today (*e.g.*, Derenko *et al.*, 2003; Pakendorf *et al.*, 2003). Continued characterisation of both modern and prehistoric Siberian populations from the Lake Baikal region and elsewhere may further illuminate the place of the Serovo-Glazkovo in the population history of Siberia. An intriguing association is seen however, between the Serovo-Glazkovo and the Xiongnu culture who used the Egyin Gol cemetery located immediately south of Lake Baikal in the Selenga river basin. The similar mtDNA haplogroup distributions observed between the Serovo-Glazkovo and the Xiongnu suggests that the population structure of the Lake Baikal region was reasonably stable from at least 4200 BC through to the third century A.D. This statement may be strengthened with the planned analysis of Y-chromosome polymorphisms (*e.g.*, Schurr *et al.*, in progress) and additional characterisation of both mtDNA and Y-chromosome lineages in other cemetery populations from the Cis-Baikal region. Ideally, additional cemetery populations would be selected to represent various temporal periods so that a comprehensive picture of the populations occupying Siberia through to modern day might be created.

All the data generated in this study will be integrated with other lines of evidence generated by the research concentrated within the Baikal Archaeology Project (*e.g.*, Weber *et al.*, 2002) to further enhance concepts explored in this study. For example, nitrogen, oxygen and strontium isotope datasets from bone and teeth are being constructed to explore whether the movements of the prehistoric Cis-Baikal groups can be traced from birth through the last years of life. Paired with DNA data, the isotopic signatures may help identify patterns of gene flow between prehistoric groups in addition to population structure within groups. Climatic modelling and the reconstruction of the Cis-Baikal environment during the Neolithic is also being undertaken to see if environmental change during the 7th millennium B.P. created circumstances contributing to the observed biological hiatus in the region.

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Chapter 5: A Tale of Two Cemeteries: Exploring Relationships Between Mortuary Ritual and Biological Affinities in Neolithic Lake Baikal Hunter-Gatherer Populations

Note of co-authorship

Mr. Vladimir Bazaliiski contributed significantly to this paper in that he was responsible for the excavation and analysis of the archaeological material from Lokomotiv and Ust-Ida. Dr. Andrzej Weber is acknowledged for contributing the radiocarbon dating data.

Introduction

Cemeteries potentially hold a wealth of information about the social and biological organisation of the communities who used them. For over a century, mortuary archaeologists and biological anthropologists have evaluated variation in mortuary practices to deduce behaviour governing kinship affiliations, gene flow and elite status in prehistoric communities (*e.g.*, Binford, 1971; Saxe, 1971; Tainter, 1976; 1978; Alekshin, 1983; Johnson and Lovell, 1994; Prowse and Lovell, 1996; Larsen, 1997). A principal objective of the Baikal Archaeology Project (BAP) has been to create a comprehensive snapshot of prehistoric Siberian hunter-gatherer behaviour through examination of their mortuary remains (*e.g.*, Link, 1996; Katzenberg and Weber, 1999; Weber *et al.*, 2002; Titurin and Bazaliiski, 1996; Bazaliiski, 2003; Bazaliiskiy and Savelyev, 2003). To date, this initiative has succeeded in illuminating an approximate 800-year biological hiatus in the Cis-Baikal region of Southeastern Siberia (Weber *et al.*, 2002). Stable isotope signatures produced for the two groups nesting the perceived hiatus suggested that they had differing subsistence strategies and mobility patterns (Katzenberg and Weber, 1999; Weber *et al.*, 2002). When these observations are combined with observed differences in mortuary behaviour, BAP scholars believe that these two groups, known as the Kitoi and Serovo-Glazkovo, were not biologically continuous groups.

In a previous study, (Chapter 4), we estimated the biological distance between a Kitoi and Serovo-Glazkovo community by analysing mtDNA RFLP and HVI polymorphisms from skeletal remains excavated from the Lokomotiv and Ust-Ida cemeteries respectively. The principal goals of this study were to test the hypothesis that the Kitoi and Serovo-Glazkovo were biologically continuous groups and explore whether either of these groups shared population affinities with populations inhabiting the Cis-Baikal region today. By estimating biological distances from mitochondrial DNA (mtDNA) haplogroup distributions, we were able to demonstrate that these Kitoi and Serovo communities were biologically discrete and that the Kitoi did not contribute to the subsequent matrilineal population structure of the Cis-Baikal region.

The variability in mortuary ritual observed between and within the Lokomotiv and Ust-Ida cemeteries suggests that people were buried in different ways for different reasons. Group membership, status, and marriage practices in either of these communities may have restricted gene flow, thus creating intra-population structure. If mortuary ritual was used to reflect such behaviour, it may be illuminated by seeking associations between biological affinity and archaeological data. Informative archaeological data may include the spatial organisation of a cemetery, radiocarbon dates, grave goods and the architecture of a grave. This approach has been used to evaluate whether gene flow was restricted between elite and non-elite individuals in a prehistoric cemetery populations (*e.g.*, Johnson and Lovell, 1994; Prowse and Lovell, 1996; Hemphill, 1999).

Traditionally, questions about biological affinity in cemetery populations have been undertaken using either cranial or dental non-metric traits (*e.g.*, Johnson and Lovell, 1994; Prowse and Lovell, 1995; 1996; Larsen, 1997). With the emergence and continued improvement of ancient DNA (aDNA) techniques (*e.g.*, Kaestle and Horsburgh, 2002), it has become possible to use DNA data to conduct biological affinities analyses of ancient cemetery populations. Indeed, DNA data may depict biological relationships between individuals with greater certainty due to the multigenic and epigenetic influences that are known to affect the expression of certain morphological traits (Larsen, 1997: p.303; Gelehrter *et al.*, 1998). However, most aDNA studies of cemetery populations to date have refrained from examining biological data within the mortuary context. Instead, these studies have focused solely on whether the aDNA data can be used to estimate biological distances existing between ancient and modern groups who inhabited the same geographic region over disparate temporal boundaries (*e.g.*, Carlyle *et al.*, 2000; Stone and Stoneking, 1998; Kaestle and Smith, 2001).

This study will assess the feasibility of using mtDNA markers to illuminate the presence of intra-population biological structure in cemetery populations. This analysis also provides the opportunity to gain new insights and understanding of the cultural behaviour in the Kitoi and Serovo-Glazkovo groups represented by the Lokomotiv and Ust-Ida cemeteries. This will be done by evaluating mtDNA haplogroup data, obtained through both restriction fragment length polymorphism (RFLP) analysis and hypervariable region I (HVI) sequencing against various lines of contextual evidence. This mtDNA data will be compared against spatial, temporal and burial indicators to seek statistically significant associations between biological affinity and mortuary practice. If any associations are identified, it may be possible to deduce whether matrilineal affiliations in the Kitoi and Serovo-Glazkovo influenced mortuary behaviour.

Materials and Methods

Cemetery descriptions

Lokomotiv

The Lokomotiv cemetery is the largest Neolithic cemetery known to exist in North Asia and was initially discovered in 1897 during construction of the Trans Siberian Railway (Ovchinnikov, 1904; cited in Bazaliiskiy and Savelyev, 2003). Radiocarbon (^{14}C) dates obtained with AMS methodology (Isotrace, University of Toronto) place the use of Lokomotiv between ca. 7250 and 6040 years before present (BP). The total area of Lokomotiv is estimated to be approximately 50 000 m^2 (Bazaliiskiy and Savelyev, 2003) and is situated on a promontory at the junction of the Irkut and Angara rivers, approximately 70 km downstream of Lake Baikal.

A site map for Lokomotiv is shown in **Appendix 6**. A total of 124 individuals from 71 graves have been excavated to date. The shaded grey area represents the portion of the cemetery that remains unexcavated. Individuals from sectors 2, 4, 5, 6, and 7 were sampled for aDNA analysis in this study.

Ust-Ida

The Ust-Ida cemetery, which was used by the Serovo-Glazkovo, is situated approximately 100 km downstream of Lokomotiv on the Angara river. Okladnikov was the first to report the discovery of Ust-Ida in 1956; however, the cemetery was not comprehensively excavated until 1987 when dam pressure downstream of Ust-Ida threatened to wash away the burials (Titurin and Bazaliiski, 1996; Bazaliiski, 2003). The Ust-Ida site map is presented in **Appendix 7** and shows two clusters of burials. Unlike Lokomotiv, Ust-Ida was excavated entirely, revealing 64 individuals in 59 graves. Individuals were sampled in similar proportions from both cemetery sectors for aDNA analysis.

Samples

Either cervical or thoracic vertebrae were sampled from 40 Lokomotiv and 42 Ust-Ida individuals for DNA extractions by a physical anthropologist conducting an osteological study in 1995 (*i.e.*, Link, 1996). Specimens for aDNA analysis were sampled in a non-probabilistic manner based on the availability of vertebrae (A. Weber, *personal communication*). Vertebrae are ideal for DNA analysis from skeletal remains as cancellous bone is expected to yield higher concentrations of DNA per unit mass of tissue than cortical bone (Lee *et al.*, 1991).

Based on macroscopic examination, the skeletal remains from both cemeteries appear to be well preserved. The grave pits at Lokomotiv were generally dug to depths exceeding 50cm into the lower levels of reddish brown loam corresponding to the Holocene climatic optimum of the Atlantic period (Bazaliiski, 2003). Many of the grave pits at Ust-Ida were dug to the upper levels of the reddish brown loam and backfilled with stones and pebbles. The climate of the Lake Baikal region is continental with warm summers, cold winters and limited precipitation; regions of discontinuous permafrost have also been noted in the area around Lake Baikal (Weber *et al.*, 2002) but not in the Angara valley. The combination of neutral pH, deep burial, cool temperatures and limited moisture favours the preservation of DNA within the osteological material.

Specimen preparation and DNA extraction

Vertebral specimens were prepared for DNA analysis using protocols described in detail in previous studies (Mooder *et al.*, 2003; Mooder *et al.*, in preparation.). Briefly, exogenous, contaminant DNA was removed from vertebral samples using combined strategy of surface removal, bleach immersion and UV treatment (*e.g.*, Ou *et al.*, 1991; Prince and Andrus, 1992). The vertebrae were rendered brittle with liquid nitrogen then pulverised using sterile mortars and pestles. All samples were extracted using a modified guanidium thiocyanate protocol (*i.e.*, Mooder *et al.*, 2003) first proposed by Boom and others (1990).

mtDNA RFLP and HVI sequence analysis

MtDNA haplogroups for individuals at Lokomotiv and Ust-Ida were identified by PCR using primers listed in **Appendix 3** flanking the major Asian-specific mtDNA restriction fragment length polymorphisms (RFLPs) and a 176 base pair (bp) region of the mtDNA hypervariable region I (HVI) where many Asian-specific polymorphisms are found. Each 50 µL reaction mix consisted of 5 µL 10X PCR Buffer (Invitrogen), 0.2 mM of each dNTP (PE Biosystems), 1.5 mM $MgCl_2$ (Invitrogen), 200 pmol of each relevant primer, (DNA Synthesis Laboratory, University of Alberta), 15 µg of BSA (NEB), and 1.25 U of Platinum *Taq* DNA Polymerase (Invitrogen). DNA extracts were not quantitated; instead, a standard 8 µL of template was added to each reaction mixture.

All amplifications were undertaken using either a Perkin Elmer (PE) 2400 thermocycler or an MJ Research PTC Minicycler. Reaction conditions varied depending on the thermocycler used; amplifications using the PE 2400 consisted of an initial denaturation step at 94 °C for 2 minutes, followed by 40 cycles of 30 s at 94 °C, 1 min at relevant annealing temperature and 30 s at 72 °C. In contrast, amplifications using the MJ PTC consisted of an initial denaturation step at 95 °C for 2 minutes, followed by 40 cycles of 60 s at 95 °C, 90 s at relevant annealing temperature and 60 s at 72 °C. Regardless of the thermocycler used, all amplifications were completed with a final extension step of 5 minutes at 72 °C.

To evaluate the gain or loss of RFLPs, 5 units of the relevant restriction enzyme and 1 µL of 10X buffer (NEB or Invitrogen) were diluted with sterile water to a final volume of 10 µL and added to the entire volume of amplified product. All restriction digests were incubated overnight at 37 °C. The digested products were visualised using a Fluor-S Multiimager with the Quantity One software package (BioRad) after electrophoresis on 12 % polyacrylamide gels and ethidium bromide (10 mg/mL) staining.

Sequencing of a portion of the mtDNA HVI was performed using primers flanking a 176 bp region of HVI from positions 16191 to 16367; the sequences for these primers are also listed in **Appendix 3**. This fragment was targeted because it contains a majority of the informative polymorphisms characterising Asian HVI variation. The primary sequencing PCR reaction reagent concentrations and conditions were the same as those used for RFLP amplifications on the MJ PTC except that 50, rather than 40 cycles of PCR were performed. The primary PCR products for cycle sequencing were purified using the QuickStep 2 PCR Purification kit (Edge Biosystems). Sequencing of 75 ng of template was undertaken for both the H and L strands using an ABI 377 sequencer and the BigDye terminator package (Applied Biosystems) following standard manufacturer specifications. The resulting sequence data were read manually and deviations from the Cambridge reference sequence (Anderson *et al.*, 1981) were noted.

Molecular sexing

To determine the sex of subadult skeletal remains interred at Ust-Ida, DNA sexing was attempted using PCR to amplify the X and Y copies of the amelogenin locus using primers originally described by Mannucci and colleagues (1994). A six bp deletion on the X-copy of the amelogenin gene allows males and females to be discriminated; females will have a single 106 bp copy, while males will produce both a 112 and 106 bp band. Amelogenin analysis has been successfully applied in identifying the sex of human remains in both forensic (*e.g.*, Gill *et al.*, 1994) and archaeological contexts (*e.g.*, Gotherstrom *et al.*, 1997; Meyer *et al.*, 2000). Similar to the approach of Vernesi *et al.*, (1999), PCR amplifications of 65 cycles were undertaken using 50 μL reaction mixtures composed of 6 μl 10x PCR Buffer (Invitrogen), 20 μg BSA (New England

Biolabs), 2.5 mM $MgCl_2$ (Invitrogen), 0.2mM of each dNTP (PE Biosystems), 200 pmol of each primer (DNA Synthesis Laboratory, University of Alberta), and 2U of Platinium Taq polymerase (Invitrogen). Amplified products were visualised as noted above for the mtDNA RFLP analysis. Adult sex determinations used in this study were obtained previously (Link, 1996; A. Lieverse, personal communication., 2003) using standard morphological approaches. The entire set of molecular sex data produced in this study can be found in **Appendix 9**.

Contamination control

All pre- and post-PCR manipulations on the samples analysed in this study were undertaken in physically separated rooms. The laminar flow cabinet where all pre-PCR sample manipulations were undertaken was decontaminated with undiluted bleach[4] prior to each use (*i.e.*, Prince and Andrus, 1992). All supplies and reagents used in this study were rendered DNA-free using methods suitable for the material being treated. All racks, pipettors, and containers were treated with undiluted bleach prior to use. Pipette tips and tubes were autoclaved in small batches. Reagents were exposed to UV light to denature any contaminant DNA (e.g., Ou *et al.*, 1991); those reagents known to degrade when exposed to UV irradiation were autoclaved instead. Both extraction and negative (no template) PCR controls were used to detect the presence of systematic contamination. Positive control material was added to PCR reaction vessels only in the post-PCR area. To control for spurious contamination, all samples were extracted in duplicate and PCR amplifications from each extract were executed over multiple events.

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[4] industrial strength bleach containing 12% w/v sodium hypochlorite.

Evaluation of authenticity

DNA derived from human skeletal remains is highly susceptible to interference from contamination and chemical modification (*e.g.*, Paabo, 1989; Paabo *et al.*, 1990; Lindahl, 1993; Kolman and Tuross, 2000; Cooper and Poinar 2000; Hofreiter *et al.*, 2001; Gilbert *et al.*, 2003); thus, retrieved aDNA data are expected to satisfy a number of criteria to be considered authentic. At a minimum, these criteria require that ancient mtDNA data be reproduced from at least two independent extracts and more than one amplification event. It is also expected that retrieved ancient mtDNA data reflects the population under study (*i.e.*, Asian polymorphisms from Asian populations) and possess variants characteristic of a given mtDNA haplogroup motif. Furthermore, recovered aDNA data are expected to differ from the mtDNA haplogroup motifs defining those individuals involved in the excavation or analysis of the human remains considered in the study; aDNA data matching that of reference samples is likely to be non-authentic. Finally, duplication of aDNA results by an independent laboratory increases the confidence in data authenticity.

Results from this study were only considered authentic if reproduced from a minimum of two independent extractions and multiple amplification events. On average, eight concordant RFLP results were obtained from each individual, thus ensuring the authenticity of the RFLP haplogroup assignments. HVI sequencing was used to screen samples for contamination derived from curation and analytical activities (Mooder *et al.*, in preparation); aDNA extracts with sequences matching those of reference samples were eliminated from the study. Samples with HVI polymorphisms not typical of a given haplogroup motif were also considered to be non-authentic, as were sequences with non-reproducible heteroplasmy. HVI sequencing was performed by a different laboratory and thus serves to corroborate the authenticity of the RFLP data.

Statistical analysis

To test null hypotheses concerning associations between biological affinity and mortuary practices, exact tests of population differentiation (*i.e.*, Raymond and Rousset, 1995) were computed using the *Arlequin* 2.000 statistical package (Schneider *et al.*, 2000). This exact test is considered to be analogous to a Fisher's exact test with a two-by-two contingency table expanded to a table of a size defined by the number of burial types and the number of haplogroups examined in this study. An exact test approach is preferable when dealing with small sample sizes as the chi-square test for independence assumes that any given cell has a minimum frequency of five (Gould and Gould, 2002). When associations could be tested using a two-by-two table, the Fisher's exact test algorithm was computed using the SYSTAT 10.2 statistical package.

Results and Discussion

Rationale

Examining the relationship between biological affinity and mortuary ritual requires that both the biological and archaeological data characterising a cemetery community be examined extensively. As neither the spatial, typological, nor chronological data were available at either Lokomotiv or Ust-Ida prior to this study's undertaking, a proportion of this paper is dedicated to these lines of archaeological evidence. Since it has been determined that the communities who used these two cemeteries were biologically discrete, each cemetery will be discussed separately.

Lokomotiv

Lokomotiv matrilineal structure

As reported previously (*i.e.*, Mooder *et al.*, 2003; Mooder, Chapter 4), authentic mtDNA RFLP haplogroup data have been retrieved from 31 of 40 (78%). Kitoi individuals from Lokomotiv. Subsequent mtDNA sequencing has characterised 6 unique HVI lineages in 10 Kitoi individuals. **Table 5-1** shows the matrilineal structure of the Kitoi who are characterised by a large frequency of haplogroup F and a combined frequency of haplogroup F and D which exceeds 70 % (Mooder *et al.*, 2003; Mooder *et al.*, *in preparation*). The mtDNA structure of the Kitoi is not typical of populations inhabiting the Cis-Baikal region today (Derenko *et al.*, 2000; 2002; 2003; Pakendorf *et al.*, 2003), the importance of which was discussed in a previous paper (Chapter 4).

Table 5-1: Lokomotiv mtDNA haplogroup and HVI data

RFLP Haplogroup	n	HVI Lineages Deviations from CRS +16000	n
A	4	223 290 319	2
C	1	223 298 327	1
D	7	223	2
F	15	234A 249 304 311	2
G2a	1	223 227 278	1
U5a	2	256 270	2
Total	31		10

Interestingly, the Kitoi are also observed to have a HVI motif characteristic of haplogroup U5a whose origins are predicted to lie in the European Palaeolithic (Sykes, 1998). It seems however, that the distribution of haplogroup U5a is wider than was first believed as evidenced by its presence at Lokomotiv, in prehistoric cemeteries from northern Mongolia (Keyser-Tracqui *et al.*, 2003) and China (Oota *et al.*, 1999; Wang *et al.*,2000; Yao *et al.*, 2003) as well as several modern Siberian groups (Derenko *et al.*, 2003).

Lokomotiv spatial organisation

Spatial organisation of a cemetery is important to biological affinities studies since people may have buried their dead in discrete areas of a cemetery for different reasons (e.g. Johnson and Lovell, 1994; Prowse and Lovell, 1996; Howell and Kintigh, 1996). As noted in the archaeological context section, several discrete clusters of graves were observed at Lokomotiv (**Appendix 6**). With the exception of Sector 2, no overt spatial organisation is found within each cluster. A figure illustrating the organisation of Sector 2 graves is shown in **Appendix 8.** The long axis of the graves is generally oriented down the gentle slope of the hill. Only one obvious row can be seen in this cluster and is found at the bottom of the slope.

Lokomotiv grave architecture and distribution

Characterising grave architecture variability in a prehistoric cemetery may illuminate differential mortuary ritual marking kinship or status (Wason, 1994). While one part of the community may choose to inter their dead in double graves, another might use single graves. Many different grave types are observed at Lokomotiv and some interesting grave patterning is seen between clusters. The distribution of burial types by cemetery sector is shown below in **Table5-2**. Sectors 4, 6 and 7 contain similar proportions of single and double burials and sector 7 has the only triple burial identified at Lokomotiv. Sector 5 consists solely of single graves which are also found in abundance in Sector 2. Sector 2 also contains the entire array of communal burials observed at this cemetery.

Table 5-2: Distribution of grave types by cemetery sector

Grave Type	Cemetery Sector 2	4	5	6	7
Single	18	3	5	1	2
Double/Triple	1	2	0	1	2
Communal	6	0	0	0	1

If a subset of burials show an abundance of grave good assemblages or evidence of increased energy expenditure, it is likely that the people within held higher status in the community (Tainter, 1976; 1978; Wason, 1994). In contrast, people interred in graves with evidence of lesser energy expenditure may have had lower status in a group. Grave architecture may be considered a marker of energy expenditure (*e.g.*, Wason, 1994). All other things being equal, *per capita* energy expenditure decreases with the number of individuals found in a grave.

Regardless of grave type, people were almost always interred in an extended supine position at Lokomotiv (Bazaliiski, 2003; Bazaliiskiy and Savelyev, 2003). Individuals in double graves were either oriented in the same direction (n=9) or head to toe (n=6). While the former orientation represents male-male, female-child and male-female burials, the latter was used only for male-female pairs (Link, 1996; Bazaliiski, 2003; Bazaliiskiy and Savelyev, 2003). Triple graves often contained a male, female and child, however one was observed to hold two adult males and an adult female.

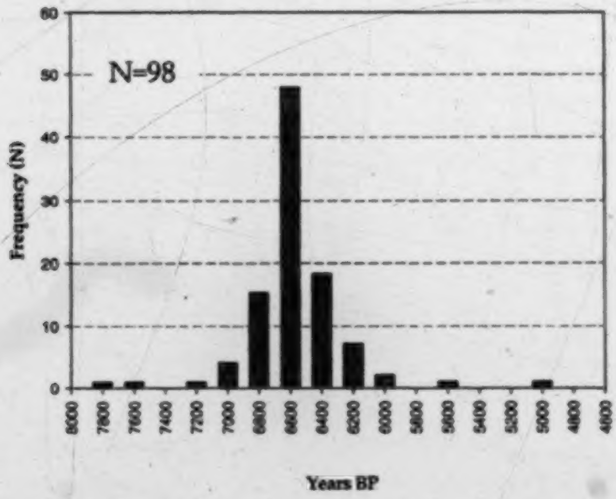
Although double and triple graves were sufficiently large for individuals to be interred side by side, the dimensions of communal graves were no larger than single graves (Bazaliiskiy and Savelyev, 2003; Bazaliiski; 2003). Thus, individuals within communal graves were stacked upon each other in pairs (Bazaliiskiy and Savelyev, 2003; Bazaliiski; 2003). While one communal grave contained only adult men (Grave 10), the remaining five held adult males and females as well as subadults who range from 3 to 18 years of age. Over 60 % of those buried in communal graves were missing crania. The likelihood that an individual has a missing skull and is found in a communal grave is statistically significant (p= 0.005). The heads of these individuals are believed to have been removed prior to burial as the vertebral columns of these individuals were positioned immediately adjacent to the margins of the grave. While three individuals in single graves were also missing skulls, artifactual assemblages left where the skull would have laid suggests that the crania were removed from the grave after burial.

Although the distribution of artifacts by grave type has yet to be comprehensively characterised for Lokomotiv, it has been observed that the most abundant artifact assemblages were found in association with the single burials, suggesting that these individuals held the highest status in the Kitoi community(Bazaliiski, 2003; Bazaliiskiy and Savelyev, 2003). In stark contrast, communal graves held only artifacts associated with violence including daggers and various points. Two individuals were found to have arrowheads lodged in their lumbar vertebrae, lending support to the idea that the communal grave individuals may have died a violent death. When this evidence of violence is considered together with the lower energy expenditure and the types of artifact assemblages, it seems likely that the individuals in communal graves were not regarded highly by those who buried them.

^{14}C Chronology at Lokomotiv

While some prehistoric communities may have used variable mortuary rituals contemporaneously, others may have changed their mortuary practices over time. To discriminate between the two, it is necessary to examine the chronology of a cemetery. The histogram representing ^{14}C dates from Lokomotiv burials in **Figure 5-1** below suggests that the Kitoi consistently used Lokomotiv for over 1200 years.

Figure 5-1: Histogram for Lokomotiv ^{14}C dates



Given the observed spatial organisation at Lokomotiv, it is important to evaluate whether the cemetery clusters were used at different times. **Table 5-3** shows the distribution of ^{14}C dates for each of the cemetery clusters. The similar distributions of dates by cluster suggest that people were buried in different sectors of the cemetery concurrently.

Table 5-3: Descriptive statistics for ^{14}C dates by cemetery cluster at Lokomotiv

	Cemetery Sector				
	2	4	5	6	7
n of Graves	25	6	5	3	4
n of ^{14}C dates	24	5	5	2	5
mean	6548	6736	6776	6713	6843
median	6615	6790	6700	6720	6815
min	5140	6380	6660	6700	6710
max	7140	6950	7250	6720	7140

As noted previously, a discrete row was observed in Sector 2 at the bottom of the slope. This row held three communal graves and one single grave. **Figure 5-2** shows the ^{14}C dates for Sector 2 burials which are organised to reflect their spatial distribution within the sector. The dates for the burials at the top of the hill are found on the far left of the figure while the dates for the discrete row are found on the far right within the box. The ^{14}C chronology for this row appears to be concentrated compared to the other burials in this sector which may suggest that these graves are somehow different. An insufficient number of individuals from this row were sampled for aDNA analysis; thus, the significance of this row can not be further characterised at this time.

Figure 5-2: ^{14}C dates for Sector 2 burials

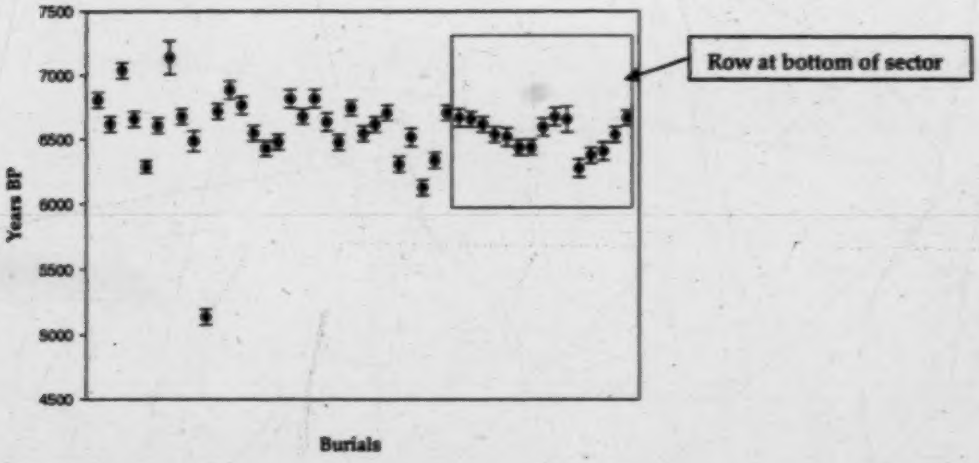


Table 5-4 shows the ^{14}C chronology by grave type at Lokomotiv. Across the cemetery, it appears that the Kitoi were using both single and communal graves concurrently. The ^{14}C dates for the double and triple graves falls within those observed for the communal and single graves; however, the absence of double and triple graves after about 6500 BP may imply that the Kitoi stopped

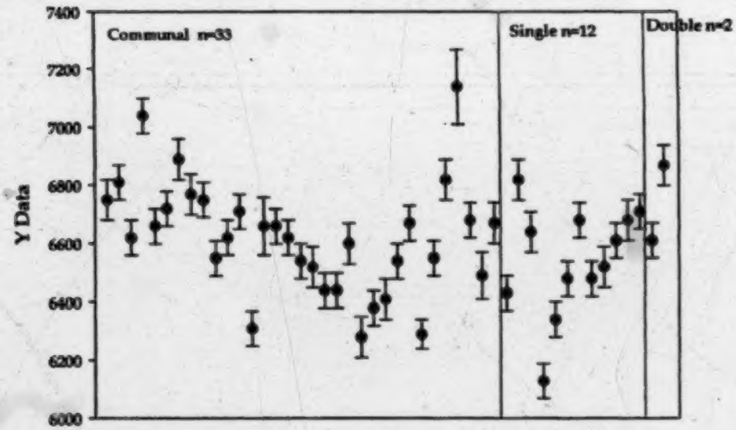
using these grave types before they stopped burying their dead in single and communal graves.

Table 5-4: Descriptive statistics for ^{14}C chronology by grave type at Lokomotiv

	Single	Double/Triple	Communal
n of ^{14}C dates	36	14	28
Mean	6645	6778	6528
Median	6670	6800	6620
Min	5700	6580	5140
Max	7250	6950	7140

Given that the communal graves were only used in Sector 2, it is also necessary to evaluate the chronological distribution of grave types within this sector. **Figure 5-3** shows the ^{14}C dates retrieved from Sector 2 individuals by grave type. As the dates for single, double and communal graves are observed to generally overlap, it seems that different circumstances governed the mortuary practice afforded those buried in this sector. Given that the Kitoi used communal graves for hundreds of years, it seems likely that behaviour associated the use of this mortuary ritual was not uncommon. The significance of this will be further explored in the context of the mtDNA haplogroup data retrieved for the Kitoi.

Figure 5-3: Chronological distribution of Sector 2 burials



Biological Affinity and Mortuary Ritual at Lokomotiv

Matrilineal affinity and spatial organisation

If the discrete clusters of graves observed at Lokomotiv reflect community units, an association between the clusters and mtDNA haplogroup distributions may suggest that the organisation of these units may have been influenced by matrilineal affinity. The use of discrete burial grounds to denote group membership within a community has been documented for several modern Siberian groups including the Nentsy, Sel'kups (Prokof'yeva, 1964a; b) and Orochi (Ivanov *et al.*, 1964a). **Table 5-5** shows the mtDNA haplogroup distributions per burial sector at Lokomotiv. Using an exact test approach in *Arlequin*, the matrilineal structure between the burial clusters is not found to significantly differ. Thus, the mtDNA data produced to date do not provide any evidence for intra-community matrilineal structure in the Kitoi. If social units within the Kitoi were organised by patrilineal affiliation, as is also documented for many contemporary Siberian groups, future examination of Y-chromosome markers may be sufficient to reveal this.

Table 5-5: MtDNA haplogroup distribution by cemetery sector at Lokomotiv

	Cemetery Sector				
Hap	**2**	**4**	**5**	**6**	**7**
A	2	1	0	1	0
C	1	0	0	0	0
D	4	1	0	1	1
F	8	2	2	1	2
G2a	0	0	0	0	1
U5a	1	1	0	0	0

Invaders at Lokomotiv?

The archaeological evidence associated with the communal graves suggests that the people within were less regarded than the remaining individuals interred in Sector 2. It has been suggested that the people in the communal graves at Lokomotiv represent an outside group (V. Bazaliiski, *personal communication*, May 2003). If this were the case, the invaders might share diminished biological affinity with the rest of the individuals buried in the sector. **Table 5-6** shows the distribution of mtDNA haplogroups by burial type for Sector 2. Haplogroups A and D are exclusive to single grave individuals. In contrast, people in communal graves have a large proportion of haplogroup F and are the only ones in the cluster to belong to haplogroup C and U5a. When these haplogroup distributions are compared using the exact test algorithm in *Arlequin,* they are found to vary significantly (P=0.002). Thus, differential matrilineal patterning suggests that the people buried in the communal graves in Sector 2 did not share notable biological affinity with the rest of the people interred in this cluster.

Table 5-6: mtDNA distribution between individuals in mass and single graves

Burial Type	mtDNA Haplogroup					
	A	C	D	F	G2a	U5a
Single	2	0	4	2	0	0
Communal	0	1	0	6	0	2

While the communal grave individuals may have been killed as a result of inter-community conflict, such a pattern may also be consistent with intra-community violence which has been noted as common practice for certain modern Siberian hunter-gatherer groups such as the Nentsy (Prokofyeva, 1964a), Nivkhi and Udegeys, (Ivanov *et al.*, 1964b; 1964c). Under this scenario, the dead in the communal graves could represent members of one Kitoi unit who were killed by the group who used Sector 2.

If the observed mortuary patterning in Sector 2 is representative of intra-community violence, the communal grave individuals should share biological affinities with other sectors at Lokomotiv. When mtDNA distributions are compared for both groups of Sector 2 graves with the other Lokomotiv clusters, no other significant differences in matrilineal patterning are identified. Thus, it seems likely that the communal grave individuals were part of the greater Kitoi community who used Lokomotiv. The origins of the communal grave people may be further illuminated by evaluating stable isotope data which have the potential to discriminate places of origin (*e.g.*, Larsen, 1997; Weber *et al.*, 2002; Weber *et al.*, 2003). Such an analysis is underway as part of the Baikal Archaeology Project. If the communal grave individuals were killed as a result of inter-community conflict, their strontium signatures may differ from the rest of the Kitoi buried at Lokomotiv. In a scenario of intra-community violence, the strontium isotope signatures of those in the communal graves should be similar to the remainder of the Lokomotiv population. If the strontium signatures are similar, further enhancement of both the mtDNA dataset as well as the characterisation of Y-chromosome markers may better describe the relationship between the communal grave people and the remainder of the population at Lokomotiv.

Elite status in the Kitoi

The differential distribution of grave goods observed for Lokomotiv burials has led Russian scholars to propose that the single burials at Lokomotiv represent the Kitoi elite (Bazaliiskiy and Saveylev, 2003; Bazaliiski, 2003). If people interred in single graves have different mtDNA haplogroup distributions than those in double and triple graves, it may be that Kitoi status was inherited along matrilineages instead of earned through the acquisition of wealth, prestige and power over a lifetime. **Table 5-7** shows the frequency of mtDNA haplogroups in single, double and triple graves at Lokomotiv. Although all but one haplogroup D individual were found in single graves, the overall distribution of mtDNA haplogroups is not found to differ significantly by burial type.

Table 5-7: mtDNA haplogroup distribution between non-communal graves at Lokomotiv

	mtDNA Haplogroups				
Burial Type	**A**	**D**	**F**	**G2a**	**U5a**
Single	2	6	4	1	1
Double/Triple	2	1	5	0	0

Further resolution of the relationships between people in single and double/triple burials is potentially obtained by comparing HVI lineages between the two burial groups as shown in **Table 5-8**. Although the HVI dataset for Lokomotiv is incomplete, several HVI lineages are found to be shared amongst burial types. Thus, if it is to be believed that single burials contain the Kitoi elite, status does not appear to have been inherited along matrilineal lines. This does not necessarily mean that Kitoi status was acquired; it could be that patrilineal affiliation dictated the inheritance of status in this prehistoric community. A comprehensive examination of the grave good assemblages at Lokomotiv is forthcoming (A. Weber, *personal communication*) and together with improved mtDNA and Y-chromosome datasets will further illuminate the question of elite status at Lokomotiv (*e.g.* Hemphill, 1999; Wason, 1994; p.93).

Table 5-8: mtDNA HVI lineages for non-communal graves at Lokomotiv

Haplogroup	HVI Motif (Deviations from CRS +16 000)	n Single	n Double/ Triple
A	223 290 319	1	1
D	223	1	1
F	232A 249 304 311	0	1
G2a	223 227 278	1	0
U5a	256 270	1	0

It may be possible to understand who was buried next to whom at Lokomotiv by evaluating biological affinities between people buried together in double and triple graves. Such an examination is limited to those burials in which all interred individuals are represented in this study. To date, only two double burials have been fully sampled for aDNA analysis. Two different patterns of double burial are observed with respect to biological affinity. People in double burials at Lokomotiv were observed to be buried in one of two ways: either in a head to toe orientation which was restricted to male-female pairs or in a side by side orientation which was observed for female-child, male-male and male-female pairs (Bazaliiskiy and Saveylev, 2003). The male-female pair for whom we have retrieved mtDNA haplogroup data were oriented head to toe. The male is haplogroup F while the female is haplogroup A.

If it can be assumed that the male-female burials in the head to toe orientation represent married couples, it might be possible to gain inferences about marriage practices in this group. For instance, if male-female pairs were never observed to share an mtDNA lineage at Lokomotiv, it may suggest that marriage between matrilineally related individuals was forbidden by the Kitoi. Similarly, marriages may have been promoted or restricted between particular Kitoi units. Evaluating associations between mtDNA, Y-chromosomal and sex data between the spatial clusters observed at Lokomotiv may illuminate a pattern for post-marital residence practices within this prehistoric community (*e.g.*, Williams *et al.*, 2002). This will be attempted in the future when the remainder of the Lokomotiv cemetery clusters are comprehensively sampled for aDNA.

The other double burial for which biological data are currently available is a male-male pair. Both individuals are haplogroup F and approximately 30–40 years of age. The similar ages and identical mtDNA lineages suggest that these two are close maternal relatives; Y-chromosome analysis will further resolve the relationship between these two individuals.

Ust-Ida

Serovo-Glazkovo mtDNA structure

As reported in previous papers, reproducible mtDNA RFLP data were retrieved from 39 of 42 (93 %) Serovo-Glazkovo individuals from Ust-Ida (Mooder *et al.*, 2003; Mooder *et al.*, *in preparation*). **Table 5-9** shows the matrilineal population structure of the Serovo-Glazkovo who are characterised by a minimum of six discrete mtDNA haplogroups and 12 unique HVI lineages. Although the Serovo-Glazkovo and the Kitoi share six mtDNA haplogroups in common, the proportions of these vary significantly (see Chapter 4). More than 50 % of Serovo-Glazkovo individuals are defined by mtDNA substitutions characteristic of haplogroups A and C. A previous aDNA study (Naumova and Rychkov, 1998) also reported mtDNA polymorphic data from a small subset of Serovo-Glazkovo individuals. However, their data have not been integrated into this study due to the differences in the mtDNA markers examined.

Table 5-9: mtDNA haplogroup distribution for Serovo-Glazkovo at Ust-Ida

RFLP Haplogroup	Frequency	HVI lineages Deviations from CRS +16000	Frequency
A	10	223 290 319	3
		223 227 290 319	2
C	11	223 298 327	2
		223 298	1
D	2	223 319	2
F	3	nd	n/a
G2a	4	223 227 262 278	1
U5a	1	256 270	1
Other*	8	223 227 262 278	2
		223	1
		311	2
		nd	3

Moderate proportions of haplogroup G2a have also been identified in the Serovo-Glazkovo which has now been comprehensively characterised in many modern Siberian populations (Derenko *et al.*, 2000; 2002; 2003). The Serovo-Glazkovo are also in possession of several authentic HVI lineages for which characteristic RFLP polymorphisms have yet to be identified. These include a lineage that is identical to haplogroup G2a except that these lineages lack the *CfoI* 7598 site loss characteristic of G2a (Kivsvild *et al.*, 2002). Like the Kitoi, haplogroup U5a is also found in the Serovo-Glazkovo, reinforcing its contribution to the prehistoric population structure of the Cis-Baikal region.

Archaeological Context

Spatial Organisation

Graves at Ust-Ida form two clusters, north and south, separated from each other by a shallow gully and a distance of approximately 25 metres (**Appendix 7**; Titurin and Bazaliiski, 1996). The long axis of the graves in either cluster is found to generally align with the Angara river which runs alongside the cemetery. The spatial patterning does not perceptibly vary between clusters. With the exception of a single, well defined row of graves found in the North Cluster, no other overt spatial organisation is observed. A comprehensive spatial analysis of this cemetery may reveal patterns that are not evident at this time.

Grave characterisation

Similar to Lokomotiv, single, double, triple and communal graves were found at Ust-Ida. The distribution of each of these grave types does not significantly differ between clusters (data not shown) and all grave types are generally observed to be interspersed amongst each other. The only exception is the single row identified in the northern cluster which exclusively holds single graves.

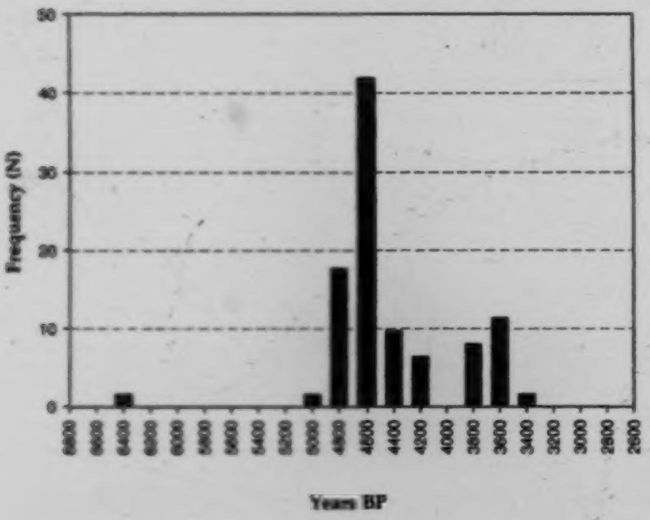
Based on body orientation, two differing burial types have been characterised for Ust-Ida. The first is the Serovo type which is identified by the orientation of the body with the head pointing upstream (or generally North). The second is the Glazkovo type and differs from the Serovo in that the head points downstream (or generally South). While Serovo individuals were buried in an extended supine position, Glazkovo burials were either supine extended or flexed on one side. Interestingly, the Glazkovo were all buried in single graves while the Serovo deposited their dead in single, double, triple and communal interments. Serovo and Glazkovo graves are observed to be interspersed amongst each other in both sectors of Ust-Ida.

The body position of double, triple and communal burials at Ust-Ida varies somewhat from that observed at Lokomotiv as individuals within are oriented in the same direction (Titurin and Bazaliiski, 1996; Bazaliiski, 2003). Similar to Lokomotiv, Serovo double graves at Ust-Ida hold different combinations of individuals including child-child (n=4), male-female (n=2), male-child (n=1), female-child (n=1) and male-male (n=1) pairs. Both the triple (n=2) and communal (n=1) graves at Ust-Ida held only subadult burials. Unlike the layered nature of the communal burials at Lokomotiv, the single communal grave at Ust-Ida contained five individuals who were deposited side by side.

Chronological Variability

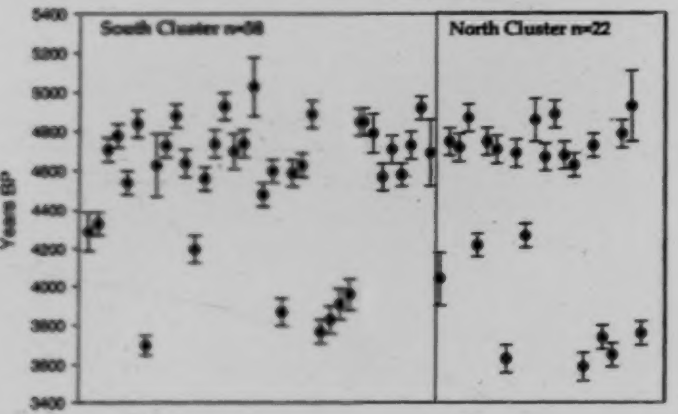
Given the spatial and typological variation observed at Ust-Ida, it is important to consider whether any associated temporal patterning could be identified. The ^{14}C dates obtained for human skeletal remains from Ust-Ida are presented in **Figure 5-4.** Overall, the chronology of dates suggests that the site was used for a time interval similar to Lokomotiv; however, temporal distribution at Ust-Ida shows an interesting bimodal pattern that wasn't evident at Lokomotiv. Ust-Ida appears to have been used frequently from ca. 5000 to 4500 BP and then more sporadically between 4300 and 3600 BP. The ^{14}C dates generally capture the expected temporal succession of the Serovo and Glazkovo mortuary complexes; however, a few graves are somewhat out of line.

Figure 5-4: ^{14}C dates for human skeletal material from Ust-Ida



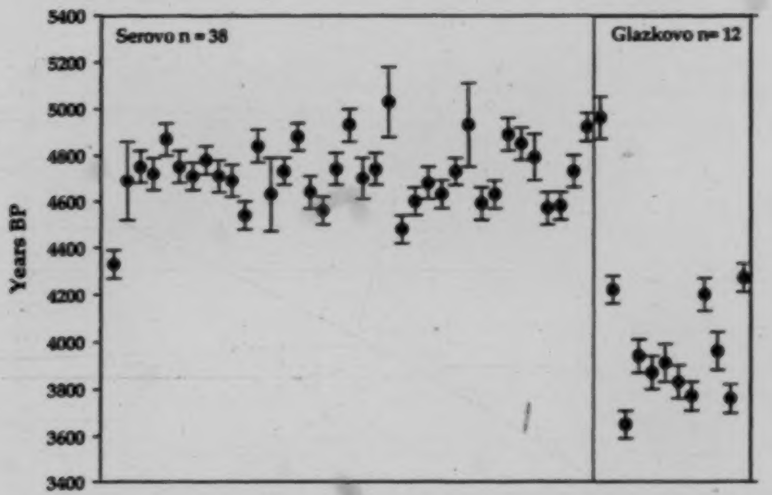
To understand whether the observed bimodality in burial chronology corresponds with the observed spatial organisation at Ust-Ida, the ^{14}C dates for the north and South Clusters of burials were compared as shown in **Figure 5-5.** The ^{14}C dates overlap for both periods of site use, suggesting that both cemetery clusters were used concurrently.

Figure 5-5: ^{14}C chronology for burials at Ust-Ida



When ^{14}C dates are instead evaluated in the context of grave typology as seen in **Figure 5-6**, the reason for the observed bimodality becomes clear. While 37 of the 38 Serovo dates fall between ca. 4500 and 5200 BP, 11 of the 12 Glazkovo dates fall into the range of 4300 to 3600 BP. Thus, an intriguing chronology of site use is revealed at Ust-Ida. First, a Serovo community used Ust-Ida for over 500 years and buried their dead with their heads pointing downstream in various types of graves. For some reason, the Serovo stopped using Ust-Ida and after approximately 200 years, a Glazkovo community started burying their dead in single graves with their heads pointing upstream. It is apparent that the Glazkovo were aware of the Serovo graves as all the Glazkovo graves were plotted between the existing Serovo graves without disturbing the physical integrity of the latter.

Figure 5-6: ^{14}C dates for Ust-Ida burials classified by typology



The relationship between the Serovo and Glazkovo is not completely understood. Cemetery use by the Serovo and Glazkovo is observed to overlap at several other sites in both the Cis-Baikal region as well as in the Lena river basin

(Weber *et al.*, 2002). The behaviour of these two groups is predicted to have been similar given their common subsistence strategies and mobility patterns. While body orientation overtly discriminates Serovo from Glazkovo burials at Ust-Ida, Serovo and Glazkovo burial typology at other cemeteries in the region is not as clear. As previously noted, Serovo and Glazkovo graves are not spatially separated at Ust-Ida. Although the material culture of the two groups is not identical, most differences are represented by evolved forms of artifacts including new pottery styles and metal objects. When all of this evidence is considered together, Weber and colleagues (2002) believe that the Serovo and Glazkovo shared more similarities than would be expected of two culturally distinct groups. By comparing the mtDNA structure of the Serovo and Glazkovo, it may be possible to illuminate to what extent these groups were also biologically distinct.

Biological affinities of the Serovo and Glazkovo

The mtDNA RFLP and HVI polymorphisms characterising the individuals found within Serovo and Glazkovo graves at Ust-Ida are presented in **Table 5-10**. The Serovo are defined by at least nine mtDNA lineages while the Glazkovo are seen to possess three. While the Serovo have a large proportion of haplogroup A (30%), the majority of Glazkovo individuals (70%) are found to be haplogroup C. When the mtDNA haplogroup distributions are compared using the exact test in *Arlequin*, the matrilineal structure of the Serovo and Glazkovo are found to significantly differ (P=0.007).

Table 5-10: mtDNA haplogroup distribution for Serovo and Glazkovo individuals

RFLP Haplogroup	Serovo n = 29	Glazkovo n = 10	HVI Deviations from CRS +16000	n Serovo	n Glazkovo
A	8	2	223 290 319	2	1
			223 227 290 319	2	
C	4	7	223 298 327	0	2
			228 298	1	0
D	1	1	223 319	1	1
F	3	0	n/o		
G2a	4	0	223 227 262 278	1	0
Other	9	0	223 227 262 278 311	2	
			256 270	1	
			311	2	
			223	1	

Although the difference in the number of mtDNA lineages defining the Serovo and Glazkovo at Ust-Ida suggests that the Serovo had a more diverse matrilineal structure than the Glazkovo, it is likely that this is an artifact of the small Glazkovo sample size. To further understand the degree to which the Serovo and Glazkovo are related, it will be necessary to characterise the mtDNA structure of the two other Glazkovo cemetery populations (*i.e.*, Kuhzhir-Nuge XIV and Kurma XI) recently excavated by the Baikal Archaeology Project as well as other Serovo-Glazkovo cemeteries located in the Cis-Baikal region.

The discovery that the matrilineal structures of the Serovo and Glazkovo at Ust-Ida are potentially different requires that we revisit our current hypothesis that the Kitoi and Serovo-Glazkovo were not biologically continuous populations (see Chapter 4). However, when mtDNA haplogroup distributions of the Kitoi, Serovo, and Glazkovo are compared, the matrilineal structure of the Kitoi is still found to significantly differ from the Serovo (P=0.00) and Glazkovo (P= 0.00). Thus, our general model for population discontinuity between pre- and post-hiatus Cis-Baikal populations is not disputed. Interestingly, relative to the Kitoi, the matrilineal structures of the Serovo and Glazkovo are not seen to differ significantly (p=0.09). Thus, it is assured that the Serovo and Glazkovo share greater biological affinity with each other than either group shares with the Kitoi.

Biological affinity and spatial organisation at Ust-Ida

If people at Ust-Ida were deliberately buried in either the North or South Cluster based on matrilineal affiliation, the comparison of mtDNA haplogroup distribution by cluster may be sufficient to reveal this. Because the Serovo and Glazkovo did not appear to use Ust-Ida concurrently, it is necessary to treat each group separately. **Table 5-11** shows the distribution of mtDNA haplogroups by cemetery cluster for the Serovo. When the statistical significance of this distribution is assessed, the Serovo burials are not observed to differ by cemetery cluster (data not shown). Within the Serovo, some interesting haplogroup distributions are worthy of noting. All haplogroup F individuals are interred in the North Cluster and are found in three separate graves. Two of these individuals were interred in double graves while the third was interred in a communal grave with four other people. Two other individuals who are haplogroup 'Other' and characterised by a single substitution at 16311 are also found in this cluster. Within the South Cluster, no individuals are observed to share HVI lineages at this time.

While there is some evidence that matrilineal affinity influenced the spatial organisation of Ust-Ida, this question will be better resolved when HVI data are produced for all individuals in the cemetery. Whether patrilineal affinity influenced where the Serovo were buried at Ust-Ida will be revealed with Y-chromosome analysis planned for the future.

Table 5-11: Spatial distribution of Serovo mtDNA haplogroups at Ust-Ida

	North Cluster n=17		South Cluster n=12	
Haplogroup	n Hap	HVI lineages(n)	n Hap	HVI lineages (n)
A	4	223 227 290 319 (2)	3	223 290 319 (2)
C	1	223 298 (1)	3	n/o
D	1	223 319(1)	0	n/a
F	3	n/o	0	n/a
G2a	2	n/o	2	223 227 262 278 (1)
U5a	1	256 270 (1)	0	n/a
Other	5	223 227 262 278 (1) 311 (2) 223 (1)	3	223 227 262 278 (1)

Table 5-12 shows the distribution of mtDNA haplogroups by cemetery cluster for the Glazkovo. Similar to the Serovo, Glazkovo mtDNA haplogroups are not observed to significantly vary by cemetery cluster (data not shown). When mtDNA HVI lineages are compared, no difference is seen suggesting that matrilineal affiliation in the Glazkovo did not determine which cluster people were interred in.

Table 5-12: Spatial distribution of Glazkovo mtDNA haplogroups at Ust-Ida

	North Cluster n= 6		South Cluster n= 4	
Haplogroup	n Hap	HVI lineages(n)	n Hap	HVI lineages (n)
A	1	n/o	1	223 290 319 (1)
C	4	223 298 327 (1)	3	223 298 327 (1)
D	1	223 319 (1)	0	n/a

As previously noted, little evidence of deliberate spatial organisation is observed in either the North or South Cluster of graves at Ust-Ida. The sole exception is the presence of a single, well defined row of graves at the northern end of the North Cluster. This row contains seven single graves of which six are Serovo and one is Glazkovo. Two of the Serovo burials are adult females, one is a subadult male and two are adult males; the remaining Serovo burial was a subadult whose sex could not be determined as this individual was not sampled for aDNA analysis. Four mtDNA haplogroups were identified in the four individuals sampled for aDNA analysis. Thus, no individual in this row shared a maternal relationship.

Interestingly, the only two elite graves at Ust-Ida are in this row. The low number of elite burials at Ust-Ida corroborates the belief that the Serovo-Glazkovo were a relatively egalitarian group (Weber, 1995; Link, 1996; Titurin and Bazaliiski, 1996; Weber *et al.*, 2002; Bazaliiski, 2003). Both these graves hold Serovo males found in association with unique assemblages of artifacts. While one of these men was haplogroup C, the other was haplogroup G2a; thus, these two men were not maternally related. Whether any of the males in this row shared a patrilineal relationship will have to await Y-chromosome analysis. The sample size of elite individuals at Ust-Ida is insufficient to make any statements about how status was granted in this group. However, when elite members in cemetery populations are not found to share biological relationships, it suggests that status was acquired rather than inherited in a group (*e.g.*, Johnson and Lovell, 1994; Prowse and Lovell, 1996).

Biological affinity and Ust-Ida double interments

Similar to what was observed at Lokomotiv, double graves at Ust-Ida held many different combinations of people. Double graves were always typologically classified as Serovo and were similarly distributed between the North and South Clusters. Within the North Cluster, two double burials were noted. A double grave (Grave 16) on the west side of the North cluster contained two mature men who were interred side by side. Although the archaeological context suggests that these two individuals were interred contemporaneously this cannot be definitively confirmed, as radiocarbon dates have not been obtained for both individuals. One of these males was haplogroup F while the other was characterised as haplogroup 'Other' with a single HVI transition at np. 16311. Thus, these two men did not belong to the same matrilineage. Interestingly, the haplogroup 'Other' male shares his unusual mtDNA lineage with a young man found in a single burial (Grave 33) in the discrete row found to the north of this grave.

The second downstream double burial (Grave 14/18)[5] contained an adolescent female (No. 18) whose cause of death appears to be associated with the large arrowhead found in her chest cavity (Titurin and Bazaliiski, 1996; Bazaliiski, 2003). In same pit, layered approximately 20 cm above her was a young male individual (No. 14) between 16 and 19 years who was observed to be in a supine orientation with his legs thrown back over his chest. The radiocarbon dates of 4750 ± 70 years for the male and 4690 ± 70 years for the female are seen to overlap, thus, suggesting that these two individuals were interred within a relatively short span of time. While grave goods were found associated with the female, none were found with the male. The male belongs to haplogroup F which also characterises two other individuals buried in the downstream cluster while the female is the only haplogroup U5a individual at Ust-Ida. Thus, the relationship between these two burials is not evident; given the lack of associated grave goods found with the male, one might speculate that he was somehow implicated in the death of the female. However, it is also likely that the unusual body position of the male resulted from a postmortem disturbance of this grave (A. Weber, *personal communication*).

Several double burials were also observed in the upstream cluster at Ust-Ida. Grave 55 contains an adolescent male, estimated to be 15 to 18 years old and a young male child between 2 and 4 years old. Radiocarbon dates are not available for either of these individuals but again, the contextual evidence suggests that they were interred contemporaneously (Bazaliiski, 2003). The adolescent male was identified as haplogroup G2a while the young child harbours polymorphisms characteristic of haplogroup C. The different mtDNA haplogroups observed in this male-male pair may be representative of a father-child pattern as the child would have inherited his mtDNA haplogroup from his mother who is not accounted for in this cemetery.

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[5] Although double graves are assigned a single number by convention, an excavation technicality resulted in the assignment of two numbers to this double grave.

The second double burial (Grave 20) contains a male and female who are both estimated to be between the age of 18 and 25. While the male haplogroup C, the female is defined by haplogroup 'Other' lineage with the 16223 16227 16262 16278 HVI motif. Just as observed in the male-female double burial at Lokomotiv, the pattern of differing mtDNA types for male and female pairs might suggest that people in these prehistoric communities did not marry those from the same matrilineage. Ethnographic studies have revealed similar rules to govern marriage practices in modern Siberian groups such as the Kets (Popov and Dolgikh, 1964) and the Nganasans (Popov, 1964).

Ust-Ida subadult interments

Subadults formed a large segment (54%) of the Ust-Ida cemetery population; they were found in many different types of graves including single, double, triple and communal interments. **Table 5-13** shows the subadult sexing results for Ust-Ida. Several Ust-Ida individuals were identified as male based on the presence of the 112 bp band alone. In these instances, sex was deemed authentic if the 112 bp fragment was reproduced a minimum of three times. Similar allelic dropout of the 106 bp fragment has been observed in other studies examining human skeletal remains (*e.g.*, Vernesi *et al.*, 1999; Meyer *et al.*, 2000);

however, the exact mechanism whereby this occurs is not understood. Confirmatory sexing of the males experiencing allelic dropout is underway using the Y-chromosome-specific SRY locus (*e.g.*, Santos *et al.*, 1998).

Table 5-13: Ust-Ida subadult molecular sex determination

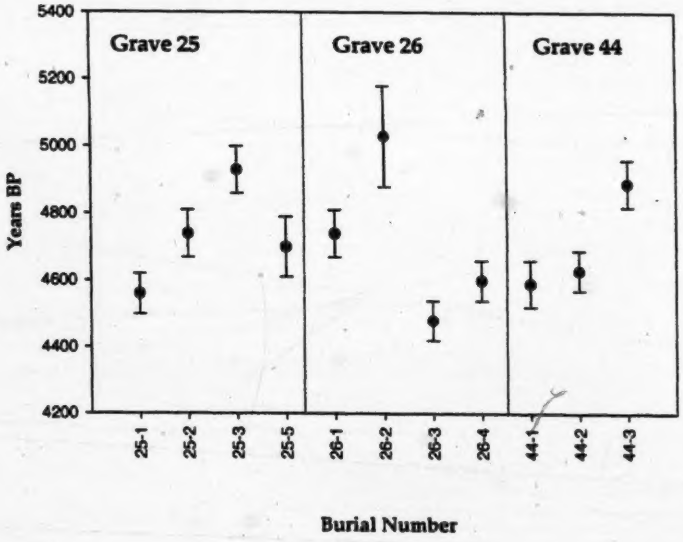
Grave Number	Grave Type	Age	Sex
5-1	Single	7–9	XY
9-1	Single	6–7	XY
26-1	Communal	13–15	XY
26-4	Communal	10–12	XY
26-5	Communal	5–7	XX
31-1	Single	10–12	XY
44-1	Triple	9–10	XX
44-2	Triple	5–6	XX
44-3	Triple	11–12	XY
53-2	Double	4–6	XY
55-1	Double	2–4	XY

The two subadult single graves were both found in the North Cluster but were not located in close proximity to each other. Both of these graves were Serovo in typology and held young males. The individual in grave 5 was between 7 and 9 years of age and haplogroup A, while the one in grave 9 was between 6 and 7 years of age and haplogroup 'Other' with an HVI motif defined by a single substitution at 16311. One of the two subadults buried in a double grave was mentioned in the previous section to be a haplogroup C male between the age of 4 and 6 years old who was buried with a young adult male who may have been this child's father. The second subadult double grave (Gr. 53) held two individuals. The individual sampled for aDNA was approximately 4 to 6 years of age and haplogroup A while the second individual was also a subadult of undetermined sex who was approximately 10-12 years of age.

One triple and two communal graves at Ust-Ida contained only subadults and two of these graves were sampled for aDNA analysis. Grave 26 was found in the North Cluster and held five individuals ranging in age from infancy to 15 years (Link, 1996; A. Lieverse, *personal communication*). Two of the adolescents within were identified as males who were both found to have a unique haplogroup A HVI motif characterised by substitutions at 16223 16227 16290 and 16319. The third individual in Grave 26 analysed for aDNA was 5 to 7 years of age; this individuals was found to be female and had the mtDNA markers characteristic of haplogroup F. Thus, only two of these three individuals belong to the same matrilineage. Within the South Cluster, a triple grave held three subadults ranging from 4 to 11 years of age. Molecular sexing revealed two of these individuals to be female and the other a male. Two of these children have identical haplogroup A lineages characterised by substitutions at 16223 16290 16319. While the third child has RFLP markers indicative of haplogroup A, a definitive HVI sequence has yet to be produced for this individual. Regardless, it is likely that all the individuals within Grave 44 belong to the same matrilineage.

While no evidence of grave disturbance has been noted for the subadult triple and communal interments (Titurin and Bazaliiski, 1996; Bazaliiski, 2003), it is not clear whether subadults were contemporaneously deposited in these graves. The ^{14}C dates from the three subadult graves containing three or more individuals are shown in **Figure 5-7**.

Figure 5-7: ^{14}C dates for triple and communal interments at Ust-Ida



In both Grave 25 and 26, the ^{14}C dates suggest that individuals were deposited over a span of hundreds of years. The error bars (representing the 68 % confidence interval) for most of these samples are relatively compact; however the small collagen yields associated with some of these samples may allow one to question their accuracy. **Table 5-14** shows the DNA, ^{14}C dates and collagen yield data for Ust-Ida subadults. Several studies have suggested that a correlation exists between collagen preservation, ^{14}C dates and DNA retrieval (Marsh and Hedges, 2000; Gotherstrom *et al.*, 2002). Thus, it seems likely that the ^{14}C dates from Ust-Ida subadult individuals for which authentic mtDNA and molecular sex data have been retrieved are accurate. The question of whether the Serovo dedicated certain graves for the exclusive interment of subadults over hundreds of years is an intriguing one which warrants further investigation.

Table 5-14: DNA, ^{14}C and collagen yield data for Ust-Ida subadults

Burial #	RFLP Haplogroup	Molecular Sex	^{14}C Date	Error (68% c.i.)	Collagen Yield (%)
25-1	nd (not determined)*	nd	4560	60	1.4
25-2	nd	nd	4740	70	4.2
25-3	nd	nd	4930	70	0.6
25-5	nd	nd	4700	90	0.4
26-1	A	XY	4740	70	4.3
26-4	A	XY	4600	60	2.5
44-1	A	XX	4590	70	6.3
44-2	A	XX	4630	60	8.6
44-3	A	XY	4890	70	9.1

Conclusions

Prehistoric cemetery populations hold a wealth of information about the lives of past peoples. The archaeological context reveals answers to questions about various cultural aspects of a group; however biological markers contained within skeletal remains have the potential to further illuminate the cultural behaviour observed. By seeking associations between biological and archaeological data, it becomes possible to gain further insight into the aspects of the social structure of a prehistoric community that are not illuminated by examining only archaeological data.

While biological affinities analyses have traditionally been undertaken using metric and non-metric dental and cranial traits, advances in aDNA methodologies allow mtDNA and Y-chromosomal markers to be applied to answer biological affinities questions in cemetery populations with greater certainty.

To date, very few aDNA studies of cemetery populations have evaluated DNA markers in the context of intra-cemetery mortuary variability (*e.g.*, Keyser-

Tracqui *et al.*, 2003). The present study demonstrates that aDNA analysis can illuminate meaning associated with observed differences in mortuary behaviour. However, it is crucial that observed associations between DNA markers and mortuary behaviour are interpreted within other contextual data to ensure the rigour of these associations. Furthermore, the analysis of both matrilineal and patrilineal DNA markers is necessary to fully characterise associations between biological affinity and mortuary ritual.

This study has conducted a biological affinities analysis of two temporally discrete prehistoric Siberian cemetery communities using mtDNA markers. The analysis of Y-chromosomal lineages at Lokomotiv and Ust-Ida is forthcoming and when combined with the mtDNA data, will define the biological relationships between people buried in these cemeteries more comprehensively. It is also anticipated that an osteological biological affinities analysis will be undertaken in the future to evaluate whether concordance can be achieved between multiple lines of biological evidence.

A substantial amount of intra- and inter-cemetery variability was observed at Lokomotiv and Ust-Ida. By integrating mtDNA haplogroup data with a few lines of archaeological evidence, several new intriguing insights about prehistoric Siberian mortuary practice were revealed. Grave clusters at Lokomotiv were not organised along matrilineal lines. Mass graves, spatially exclusive to a single cemetery sector, were observed at Lokomotiv. These graves contained layers of individuals who were in many cases, missing skulls and lacking associated artifact assemblages. As their mtDNA haplogroup distributions differed from those in the remainder of the sector, it is likely that they were either invaders or members of a different social unit who were killed as a result of intra-community violence. Forthcoming stable isotope analysis will help illuminate their population origins.

The question of differential status at Lokomotiv was also explored by seeking associations between perceived elite and non-elite burials and mtDNA haplogroup distributions. Although it seems that status was not inherited in a matrilineal fashion in the Kitoi, the relationship between biological affinity and status will not be answered until high status burials are definitively identified at Lokomotiv and Y-chromosomal analysis is done.

Although the archaeological evidence suggests that Lokomotiv was used by a single community, it appears that two groups, the Serovo and Glazkovo, used Ust-Ida. Scholars generally believe that the Serovo and Glazkovo, who often buried their dead together in cemeteries across the region, were too similar in foraging strategies and cultural behaviour to be considered as distinct groups. However, a lack of chronological overlap between Serovo and Glazkovo graves at Ust-Ida, in addition to the observed difference in their respective mtDNA haplogroup distributions, suggests that the matrilineal population structure of Ust-Ida changed with the transition to the Glazkovo grave type. The issue surrounding the relationship of the Serovo and Glazkovo reinforces why aDNA studies of cemetery populations need to integrate archaeological evidence for interpretations of biological affinity to be sound. If the mtDNA data for Ust-Ida had been reported without regard to ^{14}C dates or typological classifications, the intriguing difference between the Serovo and Glazkovo would not have been illuminated.

While significant associations between spatial organisation and mtDNA haplogroup distribution are not found for either the Serovo or Glazkovo at Ust-Ida, there is some evidence that matrilineal affinity influenced where the Serovo buried their dead. In two instances, people with identical mtDNA haplogroups or HVI lineages were found exclusive to one sector of the cemetery. In contrast, the Glazkovo were just as likely to bury their dead in either sector at Ust-Ida.

People at Lokomotiv and Ust-Ida were buried together for many different reasons. Male-female pairs from both cemetery populations have different mtDNA haplogroups suggesting that people did not marry maternal relatives. At Ust-Ida, the Serovo buried subadults in many different types of graves. Triple and communal interments at Ust-Ida were found to hold only subadults. Matrilineal relationships may not have influenced their interment in communal graves as one held subadults belonging to two different matrilineages. The exclusive subadult graves may have been used for extended periods of time given the differing ^{14}C dates observed between individuals in some of these graves. However, subadults were also observed to be buried in double and single interments at this cemetery; thus, the rules governing the mortuary treatment of Serovo-Glazkovo children will not be evident until this question is pursued in greater detail.

An imminent objective of the Baikal Archaeology Project is to further enhance the inferences made regarding Kitoi, Serovo and Glazkovo behaviour at Lokomotiv and Ust-Ida. In addition to the enhancement of the mtDNA datasets, Y-chromosomal analysis will be undertaken by Dr. Tad Schurr at the University of Pennsylvania. The future integration of these two datasets will further characterise the relationship between mortuary ritual and biological affinity in these prehistoric Siberian communities.

To better understand the relationship between the Serovo and Glazkovo, it will be important to examine other Cis-Baikal cemeteries that were used by both groups as well as those that were exclusive to the Glazkovo. Excavations at two Glazkovo cemeteries known as Kuzhir-Nuge XVI and Kurma II were recently completed and DNA analysis is in progress. Similarly, another Kitoi cemetery known as Shamanka II is currently being excavated (Bazaliiski, 2003); Shamanka II is contemporaneous to Lokomotiv and located at the south end of

Lake Baikal. The biological affinities between these two communities will be estimated to explore whether the Kitoi who used Lokomotiv had a social network that extended outside their community.

It is also our intention to explore whether it is possible to deduce the marriage practices and post marital residence patterns for the Kitoi, Serovo or Glazkovo. It may be possible to infer whether marriage was forbidden or promoted along patrilineal or matrilineal lines by examining DNA from male and female pairs in double burials at Lokomotiv or Ust-Ida. Similarly, analogues could be sought in modern Siberian populations for which marriage practices and post marital residence patterns are known (*e.g.*, Williams *et al.*, 2002). In doing so, the variance in male and female mtDNA and Y-chromosome DNA diversities may illuminate patterns of matrilocal or patrilocal residence for these prehistoric groups.

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Chapter 6: General Discussions and Conclusions

Biologists and anthropologists have long been inspired to explore questions about the origins and identity of human remains. The advent of the polymerase chain reaction brought new opportunities to such studies as it became possible to replicate DNA from human remains *in vitro*. Since then, PCR has been used to match the DNA from the dead with the living in cases of mass disaster and acts of war. It has also become feasible to evaluate whether prehistoric cemetery populations were ancestral to modern groups living in the same region.

After the initial reports documenting the successful retrieval of aDNA from human skeletal material, it became readily apparent that its retrieval was influenced by both post-mortem modification and contamination. Post-mortem DNA damage was found to reduce both the quality and quantity of aDNA retrieved which could either inhibit DNA amplification or create non-authentic polymorphism data. It was also found that DNA contamination on skeletal material or that introduced through laboratory manipulation could out-compete the DNA endogenous to the bone during PCR. Consequently, contaminant DNA could also be misinterpreted as authentic aDNA.

To reduce the impact of DNA damage and contamination, it became necessary for aDNA scholars to understand the mechanisms of post-mortem DNA modification and how modern DNA contamination could be reliably curtailed. In doing so, it was possible to design experimental protocols to optimise the retrieval of aDNA. Understanding the environmental influences on aDNA preservation was also valuable to predict whether one could expect to retrieve authentic aDNA data from skeletal material.

The skeletal remains examined in this study represent two prehistoric groups known as the Kitoi and Serovo-Glazkovo who both lived in the Cis-Baikal region of Central Siberia thousands of years ago. These two groups buried their dead in a cold, dry environment that optimised the preservation of their skeletal material. Both the Kitoi and Serovo-Glazkovo were excavated prior to the commencement of aDNA studies and curated in a university anthropology department. As such, it was imperative to understand if curation activities impacted the retrieval of authentic DNA from these samples.

By examining a subset of samples from Lokomotiv, it was discovered that modern DNA transferred to skeletal material during osteological examination could thwart decontamination protocols and obscure the aDNA endogenous to the bone. By evaluating whether this contamination was consistent in its expression, it was possible to create a set of reliable criteria to discriminate authentic from non-authentic aDNA data for both the Kitoi and Serovo-Glazkovo. Data for both RFLP and HVI markers were only reported in this study if they were reproduced from independent extracts. In cases where both RFLP and HVI substitutions were characterised for an individual, the data were expected to make phylogenetic sense and be concordant with each other. Furthermore, data were only deemed authentic if polymorphisms characterised for Asian populations were found.

The skeletal populations examined in this study represent the largest mortuary samples of this antiquity ever to be characterised for ancient mtDNA and nDNA polymorphisms. Furthermore, the aDNA analysis of Kitoi and Serovo-Glazkovo skeletal material represent the first time the matrilineal population structure of prehistoric Siberia has been described for conventional mtDNA polymorphisms. Reproducible RFLP data were generated from 31 Kitoi individuals with an approximate average postmortem interval (PMI) of 7500 years. Initial mtDNA sequencing produced 10 authentic sequences representing seven unique HVI lineages. The Kitoi are characterised by six different mtDNA haplogroups, all of which have been identified in modern Siberian populations from both the Cis-Baikal region and elsewhere. These include haplogroups A, C, D, G2a, F and U5a. Although the origins of haplogroup U5a are predicted to be rooted in West Eurasia, its presence here and in other prehistoric and modern groups from the Cis-Baikal region provides an enhanced estimate of its distribution.

Reproducible RFLP data was retrieved from 39 Serovo-Glazkovo individuals with an approximate mean PMI of 5100 years. Initial mtDNA sequencing produced 19 authentic HVI sequences representing 12 unique lineages. The same suite of mtDNA haplogroups found in the Kitoi is also represented in the Serovo-Glazkovo. However, about a quarter of the Serovo-Glazkovo are also defined by RFLP and HVI markers representative of haplogroups that have yet to be fully identified. It is highly unlikely that these lineages represent DNA damage or contamination as they are reproducible and have been identified in various modern Siberian groups (Derenko *et al.*, 2003) as well as the Egyin Gol cemetery population (Keyser-Tracqui *et al.*, 2003) discussed in this study.

The principal objective of this study was to test the population discontinuity hypothesis proposed for Neolithic Cis-Baikal by Weber and colleagues (2002). The Kitoi and Serovo-Glazkovo had different mortuary practices, mobility patterns and subsistence strategies; all of which suggest that they did not share an ancestor-descendent relationship. When mtDNA distributions of the Kitoi and Serovo-Glazkovo were compared using statistical and biological distance algorithms, these two prehistoric communities were found to be significantly different. Thus, it is likely that the Kitoi and Serovo-Glazkovo were biologically discontinuous groups. Weber and others (2002) assumption of group size and mobility patterns for the Kitoi and Serovo-Glazkovo were also corroborated with estimates of mtDNA diversity. The moderate gene diversity estimate for the Kitoi in association with its distance away from the centroid of distribution suggests that the Kitoi were a large, reproductively isolated group. In contrast, the high mtDNA diversity of the Serovo-Glazkovo is consistent with a group experiencing high levels of migration and thus fits with the model of high mobility proposed for this group.

The mtDNA structure of the Kitoi was moderately diverse and temporally stable; thus it is unlikely that the biological hiatus observed in the Cis-Baikal Middle Neolithic occurred due to their extinction. When the variances of the mtDNA haplogroup distributions for the Kitoi and Serovo-Glazkovo are compared against those for modern Siberian and Central Asian populations, the Kitoi do not cluster with any of the South-Central Siberian groups. Instead, the Kitoi appear to share affinities with Uralic-Samoyedic groups from Northwestern Siberia; this is largely due to high proportions of haplogroup F shared by these groups. When ethnographic comparisons are sought, a few similarities emerge between the Kitoi and a group known as the Kets who inhabit the Yenisey river basin west of Lake Baikal. Not only do these groups share similar subsistence strategies and mortuary practices, ancestors of the Kets were believed to have inhabited the lower reaches of the Angara river. Thus, instead of remaining in the Cis-Baikal region, the Kitoi may have migrated towards the Yenisey river basin where they contributed to the subsequent population structure of Northwestern Siberia. Future analysis of Y-chromosomal markers may further delineate the relationships between the Kitoi and groups in this region.

Unlike the Kitoi, biological distance estimates show the Serovo-Glazkovo to generally cluster with modern groups from South-Central Siberia. The strongest affinities are seen with the Todjins who share a unique haplogroup C lineage with the Serovo-Glazkovo. However, the Todjins do not have haplogroup G2a which is found in the Serovo-Glazkovo at moderate frequencies.

The large proportion of haplogroup A in the Serovo-Glazkovo suggests shared affinities with Northeast Siberian groups such as the Chukchi and Koryaks; like the Serovo-Glazkovo, these groups also lack haplogroup B. However, the Chukchi and Koryaks also have haplogroups G, Y and Z, suggesting that these groups are not likely to be descendents of the Serovo-Glazkovo.

Although affinities between the Serovo-Glazkovo and modern Siberian groups are not overt, an interesting association with a third century B.C. northern Mongolian cemetery group (*e.g.*, Tracqui-Keyser *et al.*, 2003) suggests that the population structure of the Cis-Baikal region remained relatively stable for over four millennia. This cemetery, known as Egyin Gol, is located on a tributary of the Selenga river which drains into Lake Baikal at its southern end. The matrilineal structures of the Xiongnu who used Egyin Gol and the Serovo-Glazkovo at Ust-Ida are strikingly similar, suggesting that gene flow occurred between the female descendents of the Serovo-Glazkovo and the nomadic Hun males. The association between these two groups also reinforces the Neolithic population shift in Cis-Baikal as the Kitoi do not share any strong affinities with the group at Egyin Gol.

This study also evaluated whether biological affinities within the Kitoi and Serovo-Glazkovo could be compared against observed mortuary practices at Lokomotiv and Ust-Ida to illuminate the behaviour of these prehistoric groups. While this approach has been frequently undertaken comparing phenotypic variation of the skull and teeth, its analogous application using aDNA is relatively new.

Comparisons between ^{14}C dates and mtDNA data at Lokomotiv suggest that only one group used Lokomotiv since the Kitoi mtDNA structure remained relatively stable over the duration of this cemetery's use. Distinct clusters of graves were observed at Lokomotiv, suggesting that the Kitoi may have been organised into social units who all used discrete burial grounds. The similar distribution of mtDNA haplogroups observed between cemetery clusters indicates that if such organisation was found within the Kitoi, it was not based on matrilineal affiliation. Several graves within each cluster have yet to be sampled for aDNA analysis and when done, will more thoroughly characterise the mtDNA structure of this Kitoi community.

Within the largest cemetery cluster at Lokomotiv, differences in mtDNA haplogroup distributions between those in single and mass graves suggest biological affinity may have influenced social interactions and subsequent mortuary ritual in this Kitoi community. Archaeological evidence suggests that the mass grave individuals were less regarded than others buried in this sector given the much lower energy expenditure and tradition of crania removal associated with their burials. That the mass grave individuals share matrilineal affinities with those in the remaining cemetery sectors somewhat disputes the idea that they were invaders. Instead, the observed pattern appears to be more consistent with intra-community conflict which, according to Siberian historical and ethnographic accounts, is believed to have occurred frequently (Ivanov *et al.*, 1964a; b; Prokev'ya, 1964). If the mass grave individuals were indeed members of another community, comparing strontium isotope signatures between mass and non-mass grave individuals may be sufficient to reveal this.

Based on differences in grave good assemblages and other measures of energy expenditure, the individuals in single interments at Lokomotiv are believed to represent the Kitoi elite (V. Bazaliiski, personal communication). As individuals in these graves are seen to possess several different mtDNA haplogroups, it seems unlikely that Kitoi status was inherited along matrilineal lines. It is interesting to note however that in all but one instance, haplogroup D individuals are interred only in single graves which might reflect the deliberate use of a mortuary ritual to reflect a shared matrilineal affiliation. A substantial number of single grave individuals have yet to be sampled for aDNA and this will facilitate further insight into the question of elite status in this community.

While Lokomotiv was likely to have been consistently used by a continuous community with a stable population structure, the same does not appear to have been the case at Ust-Ida. Scholars believe the Serovo and Glazkovo to be chronologically and culturally continuous given that they are observed to share several cemeteries throughout the Cis-Baikal region and the Lena river basin (Weber *et al.*, 2002). However, the lack of chronological overlap between Serovo and Glazkovo graves at Ust-Ida together with their different mtDNA haplogroup distributions hints at a change in the Ust-Ida matrilineal structure that occurred with the transition from Serovo to Glazkovo cemetery use. However, the number of Glazkovo individuals sampled in this study is at this time too small to make any definitive statements about their relationship with the Serovo.

Given the suspected change in the matrilineal population structure at Ust-Ida, it became necessary to reassess the relationship of the Kitoi to the Serovo and Glazkovo. The mtDNA haplogroup distributions of the Serovo and Glazkovo both varied significantly from that of the Kitoi but relative to the Kitoi did not vary from each other. This observation is still consistent with the overall model of population discontinuity between pre-hiatus and post-hiatus populations in Cis-Baikal. The degree to which matrilineal affinities are shared between these three groups will be further illuminated with the future sampling of mtDNA from other Serovo-Glazkovo cemeteries throughout the Cis-Baikal region. Similar examination of Y-chromosomal polymorphisms will serve to identify if patrilineal affinities exist between Serovo and Glazkovo males.

Both the Kitoi and Serovo buried people together for a variety of reasons. At Lokomotiv, people were buried in double graves either oriented side by side or head to toe (Bazaliiskiy and Savelyev, 2003). The former orientation represented male-male, female-child and male-female combinations. Only one grave with this orientation was fully sampled for aDNA and contained two males who had the same mtDNA haplogroup, suggesting that they were maternal relatives. If future Y-chromosomal analysis reveals these two to share the same patrilineage, it seems likely that they were brothers.

At Lokomotiv, the double grave head to toe orientation was used exclusively for male-female pairs which contrasted with the side-by-side double interment used for male-male, male-female and female-female pairs; this may suggest that the head to toe double burials were used to denote those who exchanged genes in this prehistoric community. The head to toe male-female pair sampled for aDNA were found to have different mtDNA haplogroups. At Ust-Ida, all combinations of individuals in double graves were oriented in the same direction. However, just like at Lokomotiv, the male-female pair also had different mtDNA lineages. If additional aDNA sampling at both cemeteries reveals all male-female burials to have different mtDNA haplogroups, it might suggest that marriage was not permitted between maternal relatives in either of these prehistoric communities. Such sampling should also include the two triple graves at Lokomotiv that hold a male, female and child combination.

It appears that graves with more than two people at Ust-Ida were used exclusively for the deposition of subadults. Molecular sexing using the amelogenin locus revealed similar proportions of males and females to be buried within these units. While individuals in one triple grave were all found to possess the same mtDNA lineage, three individuals in a grave containing five people were found to have two different matrilineages. Thus, subadults interred together at Lokomotiv were not necessarily maternally related. It is unclear whether the individuals within these graves were interred at the same time since ^{14}C dates for some burials are found to be discrepant. However, the intricacies associated with ^{14}C dating of degraded skeletal material require that this issue be investigated in greater detail before it can be said with any certainty that the Serovo used these burials to deposit subadults from generation to generation.

This study has provided a unique glimpse into how DNA from human skeletal remains can be used to reconstruct both the population history of prehistoric communities and that of a region over thousands of millennia. The hypotheses tested in this study have revealed aspects of prehistoric Cis-Baikal existence that were accessible only by integrating archaeological and biological lines of evidence. While DNA data provided crucial evidence to support principal hypotheses explored within the Baikal Archaeology Project, archaeological evidence was the key to illuminating biological subdivision at Ust-Ida that may otherwise have remained hidden.

This strategy will subsequently be used to describe the population structure and cultural behaviour of three other Cis-Baikal cemeteries currently being examined by BAP. The aDNA datasets produced for Lokomotiv will be compared against that generated for another contemporaneous Kitoi cemetery known as Shamanka II (Bazaliiski *et al.*, 2003). In doing so, it will be possible to gain further insight into the nature of social interactions between prehistoric Kitoi communities living in the Cis-Baikal region. Likewise, the aDNA analysis of two additional post-hiatus cemeteries known as Khuzhir-NugeXIV and Kurma XI will enhance our knowledge about the relationship between the Serovo and Glazkovo.

Although these papers have described how genetic techniques can be used to answer questions about anthropological questions, the strategies and results synthesised here also have direct relevance to the medical and forensic sciences. In an era where knowledge about the human genome is continuously advancing, many medical investigators are inclined to reanalyse stored tissue samples for emerging molecular markers. Medical researchers could exploit knowledge about mechanisms of *in vitro* postmortem DNA damage to design suitable histological preservation strategies or tissue storage media to enhance the preservation of DNA. The approaches taken in this study to optimise the analysis of degraded DNA and its subsequent interpretation could be used by molecular pathologists to ensure that the molecular data retrieved from stored tissue samples are authentic.

Similarly, the aDNA strategies developed in this study are of benefit to those attempting to analyse DNA from degraded human remains. Hundreds of thousands of dead individuals lie unidentified in mass graves throughout the world. At this time, DNA identification is infrequently used by humanitarian agencies due to the prohibitive expense associated with it's application. It is my intent to use the aDNA strategies developed in this study to explore cost-effective means to identify degraded human remains from forensic contexts. By giving names to the slain, they are given dignity in death and solace is provided to their families left behind.

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Appendices

Appendix 1: Statistical software for ancient DNA population comparisons

Free Software

Arlequin	http://anthro.unige.ch/arlequin
ANTANA (Harpending and Rogers, 1984; obtained from the author with permission)	
Network	http://www.fluxus–technology.com/
Migrate	http://evolution.genetics.washington.edu/lamarc/migrate.download.html
PHYLIP	http://evolution.genetics.washington.edu/phylip.html

Commercial Statistical Packages

Systat 10.2	http://www.systat.com/
MINITAB	http://www.minitab.com/
SigmaStat v. 3.0	http://www.spss.com/sigmastat/

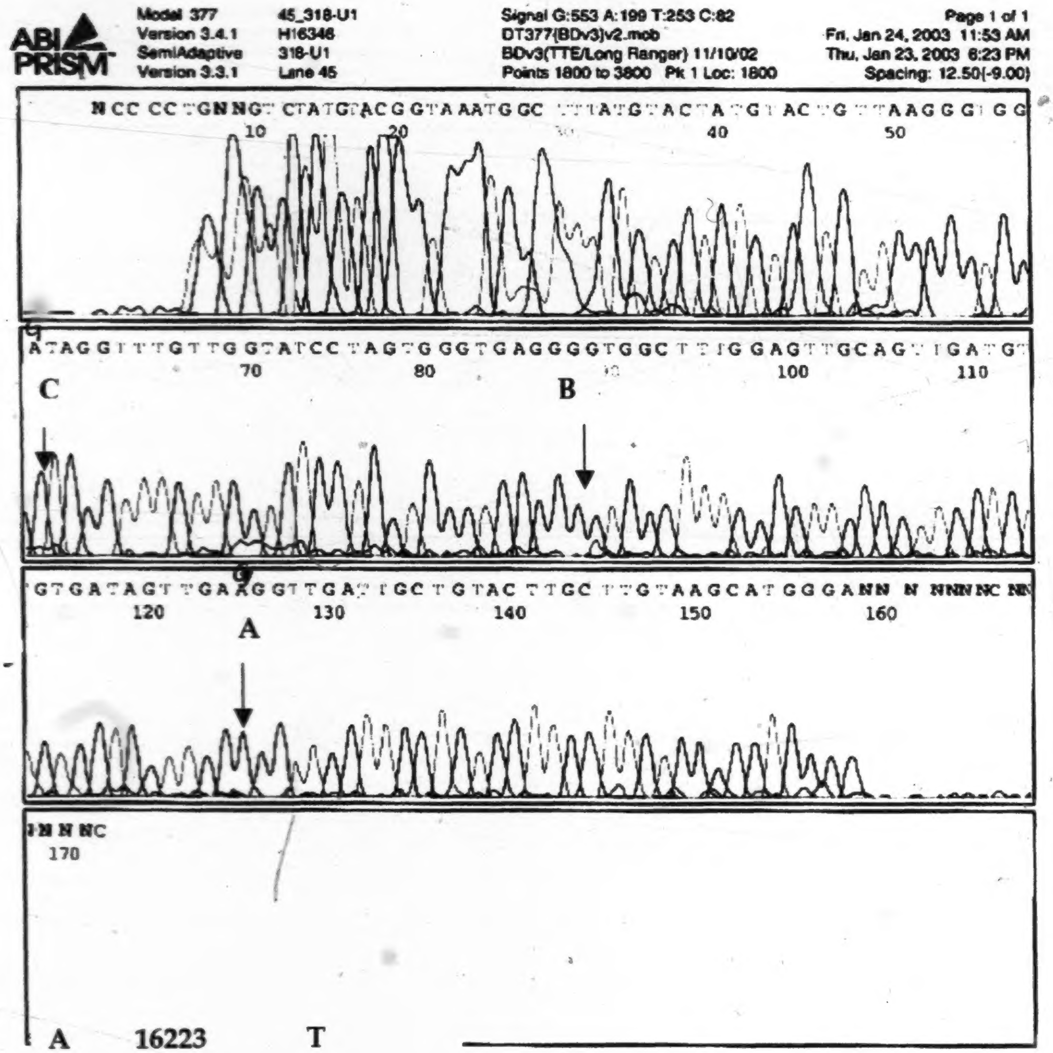
Appendix 2a: Summarised mtDNA HVI sequences from deliberately handled samples

The sample numbers with an 'F' suffix represent the samples that were fully treated, including bone surface removal. The sample numbers with a 'P' suffix belong to the samples that were only subjected to bleach and UV treatment. Samples 161 and 318 were taken from thoracic vertebrae while 586, 272 and 280 were taken from tibia, humerus and femur respectively. The sequence labelled LM corresponds to the experimental handler, KM corresponds to the analyst and DL is the osteologist who worked with this skeletal collection eight years prior to the aDNA analysis. The data table follows on the next page.

Specimen	Grave Number	16223	16224	16250	16256	16259	16290	16298	16311	16320
Cambridge (L Strand)		C	T	C	C	C	C	T	T	C
LM		T	*	*	*	*	*	*	*	T
DL		*	C	*	*	*	*	*	C	*
KM		*	*	*	*	*	*	C	*	*
161–F1	24-4-1	*	C/T	*	*	*	*	*	C	T
161–F2		*	C/T	*	*	*	*	*	C/T	C/T
161–P1		*	C/T	*	*	*	*	*	C/T	C/T
318–F1	41-2-1	*	C	C/T	*	*	*	*	C	T
318–F2		*	C	*	*	*	*	*	C	T
318–P1		T	*	*	*	C/A	T	*	*	*
318–P2		*	C	*	*	*	*	*	C	T
586–F1	24-3-1	*	C	*	*	*	*	*	C	*
586–F2		*	C	*	*	*	*	*	C	T
586–P2		*	C/T	*	*	*	*	C/T	C	C/T
272–F1	2-4-2	*	C/T	*	*	*	*	*	C/T	*
272–F2		*	*	*	T	*	*	*	*	*
272–P1		*	C	*	*	*	*	*	C	T
272–P2		*	C	*	*	*	*	*	C	*
280–F2	18-1-3	*	C/T	*	*	*	*	*	C/T	C/T
280–P1		*	C/T	*	*	*	*	*	C	T
280–P2		*	C	*	*	*	*	*	C	T

Appendix 2b: Raw sequence data for deliberately handled samples

Extract 318 P1: H strand



ABI PRISM

Model 377
Version 3.4.1
SemiAdaptive
Version 3.3.1

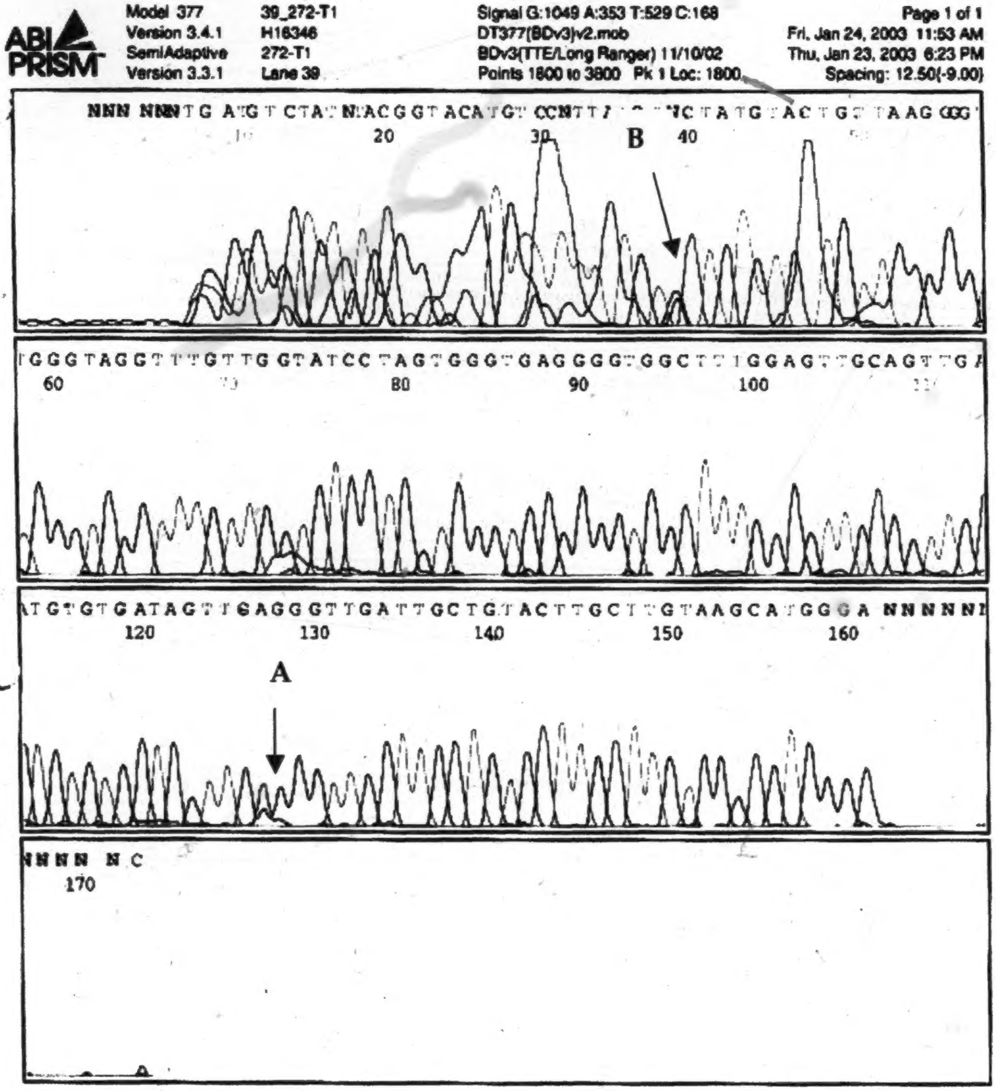
45_318-U1
H16346
318-U1
Lane 45

Signal G:553 A:199 T:253 C:82
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Fri, Jan 24, 2003 11:53 AM
Thu, Jan 23, 2003 6:23 PM
Spacing: 12.50(-9.00)

A	16223	T
B	16259	C/T
C	16290	T

Extract 272 F1: H strand



ABI PRISM

Model 377
Version 3.4.1
SemiAdaptive
Version 3.3.1

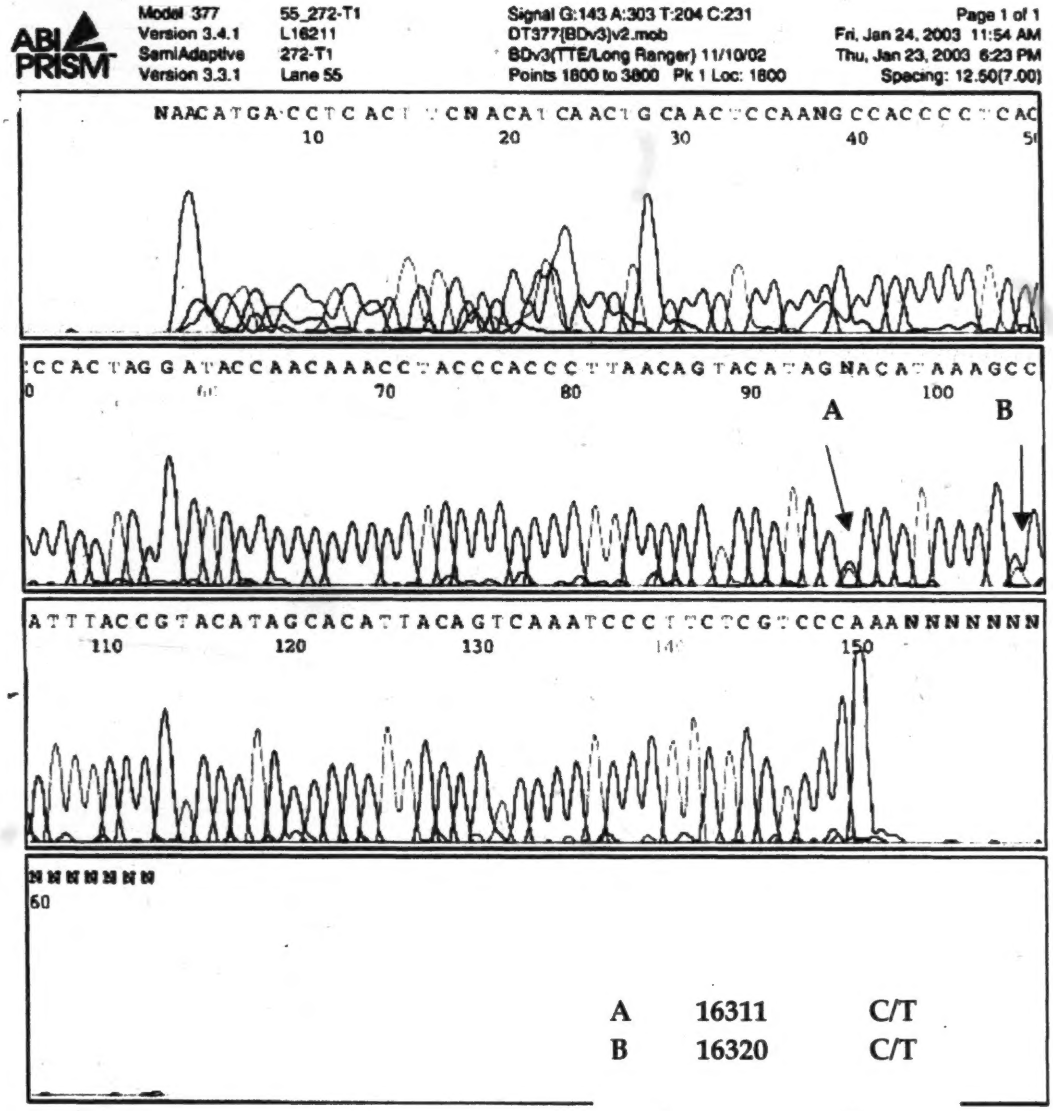
39_272-T1
H16346
272-T1
Lane 39

Signal G:1049 A:353 T:529 C:168
DT377(BDv3)v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Fri, Jan 24, 2003 11:53 AM
Thu, Jan 23, 2003 6:23 PM
Spacing: 12.50(-9.00)

A	**16224**	**C/T**
B	**16311**	**C/T**

Extract 272 F1: L strand

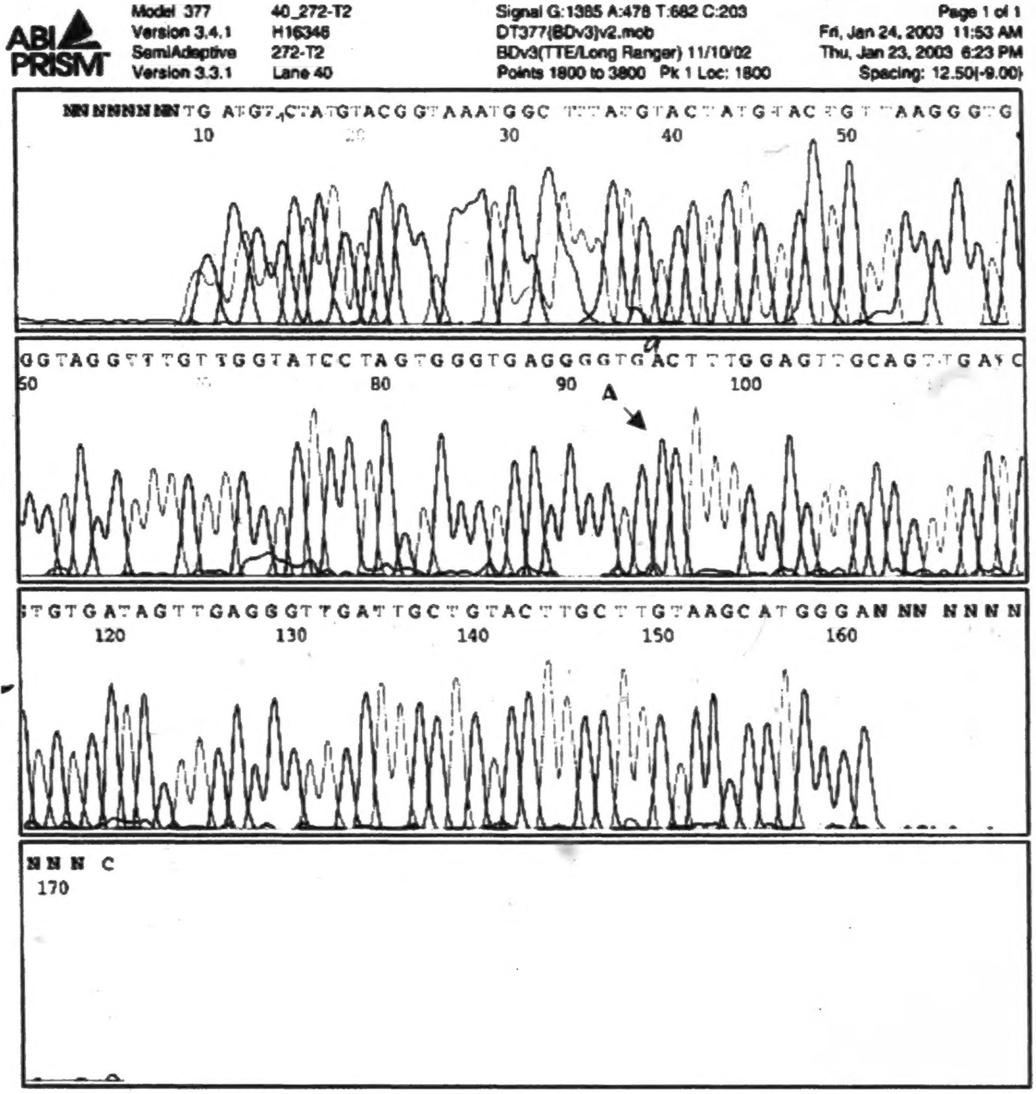


```
Model 377           55_272-T1      Signal G:143 A:303 T:204 C:231          Page 1 of 1
Version 3.4.1       L16211         DT377{BDv3}v2.mob              Fri, Jan 24, 2003 11:54 AM
SemiAdaptive        272-T1         BDv3{TTE/Long Ranger} 11/10/02 Thu, Jan 23, 2003 6:23 PM
Version 3.3.1       Lane 55        Points 1800 to 3800  Pk 1 Loc: 1800  Spacing: 12.50(7.00)
```

```
NAACATGACCTCACTTCNACATCAACTGCAACTCCAANGCCACCCCTCAC
CCACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAGNACATAAAGCC
ATTTACCGTACATAGCACATTACAGTCAAATCCCTTCTCGTCCCAAANNNNNNN
NNNNNNN
```

A	16311	C/T
B	16320	C/T

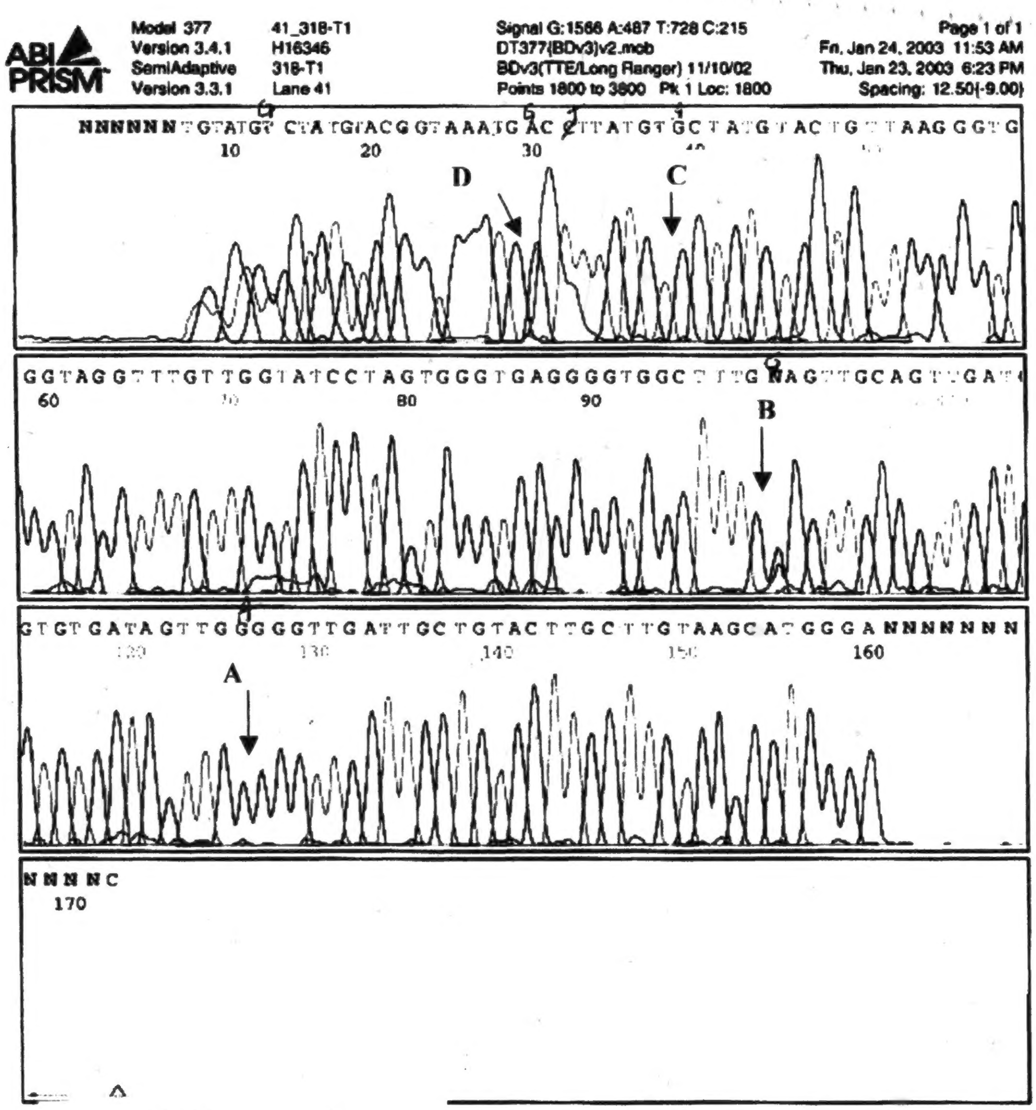
Extract 272 T2: H strand



Model 377 | 40_272-T2 | Signal G:1385 A:478 T:682 C:203 | Page 1 of 1
Version 3.4.1 | H16346 | DT377{BDv3}v2.mob | Fri, Jan 24, 2003 11:53 AM
SemiAdaptive | 272-T2 | BDv3(TTE/Long Ranger) 11/10/02 | Thu, Jan 23, 2003 6:23 PM
Version 3.3.1 | Lane 40 | Points 1800 to 3800 Pk 1 Loc: 1800 | Spacing: 12.50(-9.00)

A 16256 T

Extract 318 F1: H strand



ABI PRISM

Model 377
Version 3.4.1
SemiAdaptive
Version 3.3.1

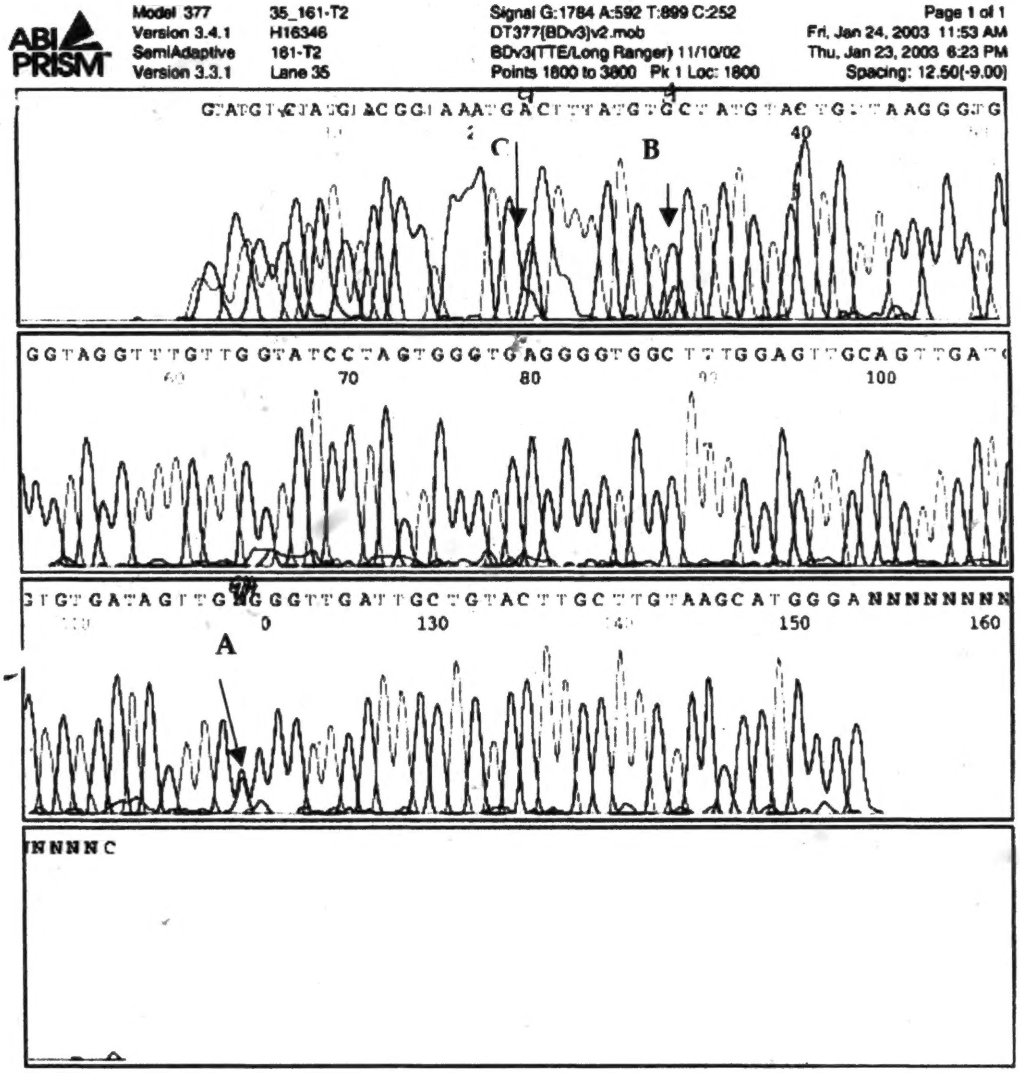
41_318-T1
H16346
318-T1
Lane 41

Signal G:1566 A:487 T:728 C:215
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Fri, Jan 24, 2003 11:53 AM
Thu, Jan 23, 2003 6:23 PM
Spacing: 12.50(-9.00)

A	16224	C
B	16250	C/T
C	16311	C
D	16320	T

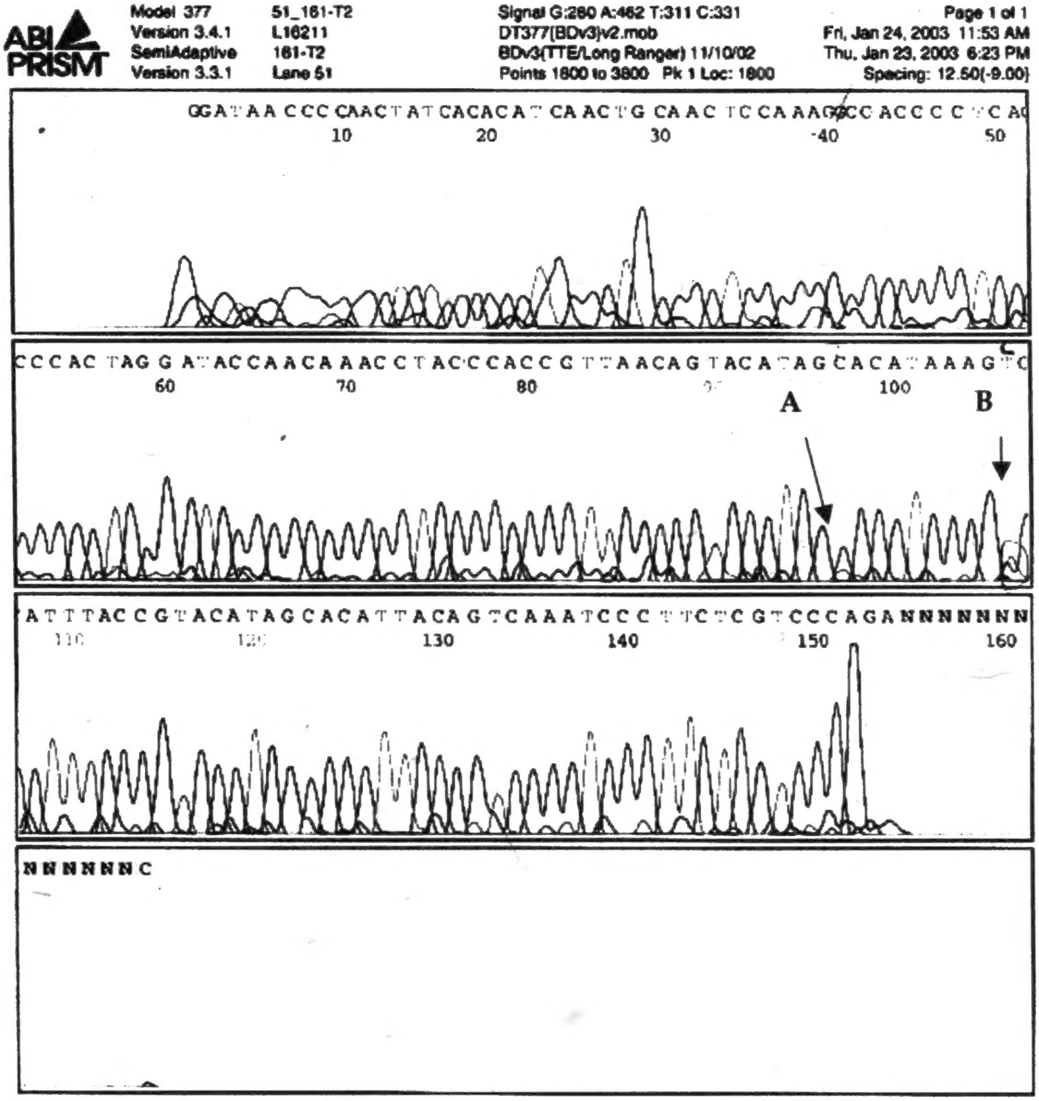
Extract 161 F2: H strand



Model 377 | 35_161-T2 | Signal G:1784 A:592 T:899 C:252 | Page 1 of 1
Version 3.4.1 | H16346 | DT377{BDv3}v2.mob | Fri, Jan 24, 2003 11:53 AM
SemiAdaptive | 161-T2 | BDv3{TTE/Long Ranger} 11/10/02 | Thu, Jan 23, 2003 6:23 PM
Version 3.3.1 | Lane 35 | Points 1800 to 3800 Pk 1 Loc: 1800 | Spacing: 12.50(-9.00)

A	**16224**	**C/T**
B	**16311**	**C/T**
C	**16320**	**C/T**

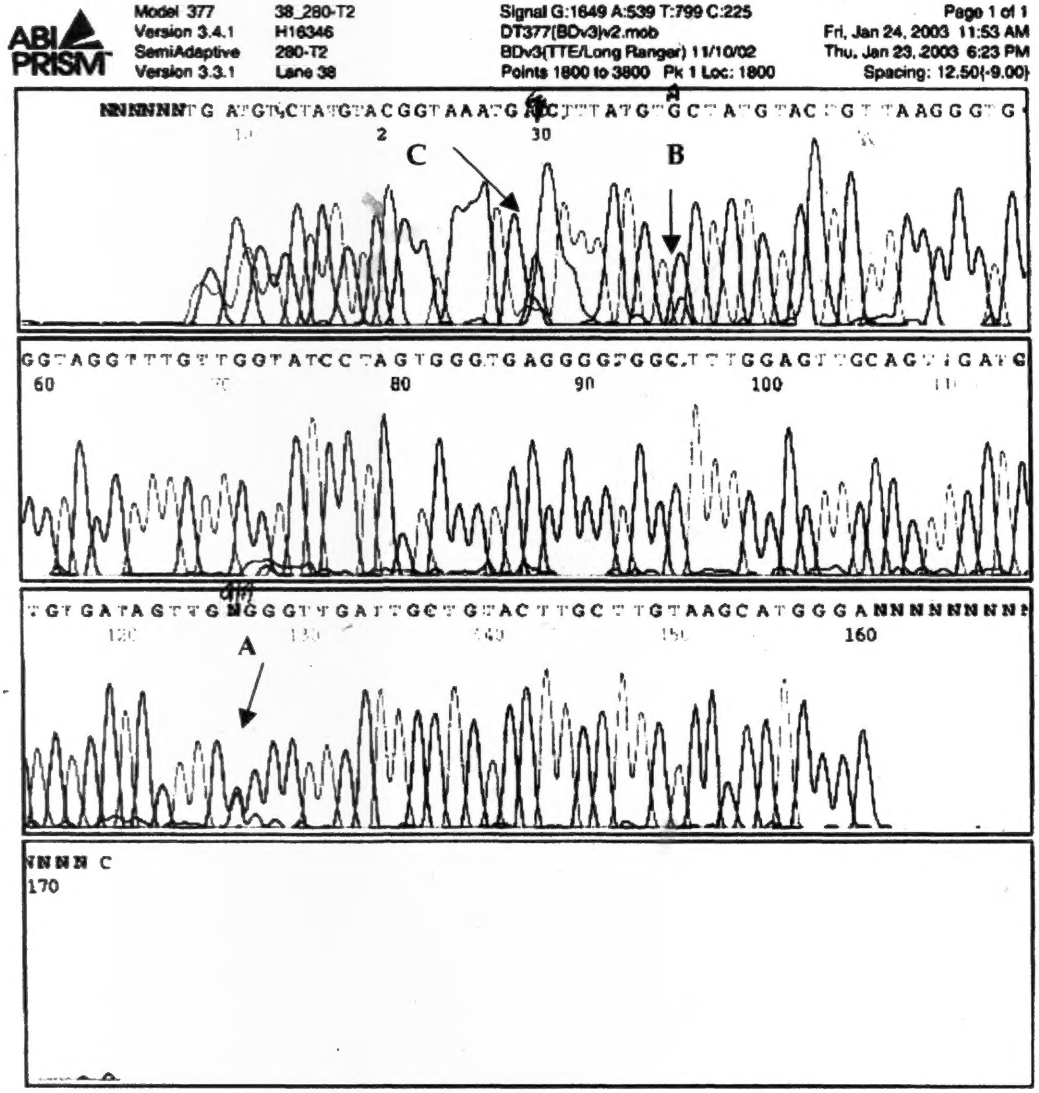
Extract 161 F2: L strand



Model 377	51_161-T2	Signal G:280 A:462 T:311 C:331	Page 1 of 1
Version 3.4.1	L16211	DT377[BDv3]v2.mob	Fri, Jan 24, 2003 11:53 AM
SemiAdaptive	161-T2	BDv3(TTE/Long Ranger) 11/10/02	Thu, Jan 23, 2003 6:23 PM
Version 3.3.1	Lane 51	Points 1800 to 3800 Pk 1 Loc: 1800	Spacing: 12.50(-9.00)

A	**16311**	**C/T**
B	**16320**	**C/T**

Extract 280 F2: H strand



Model 377
Version 3.4.1
SemiAdaptive
Version 3.3.1

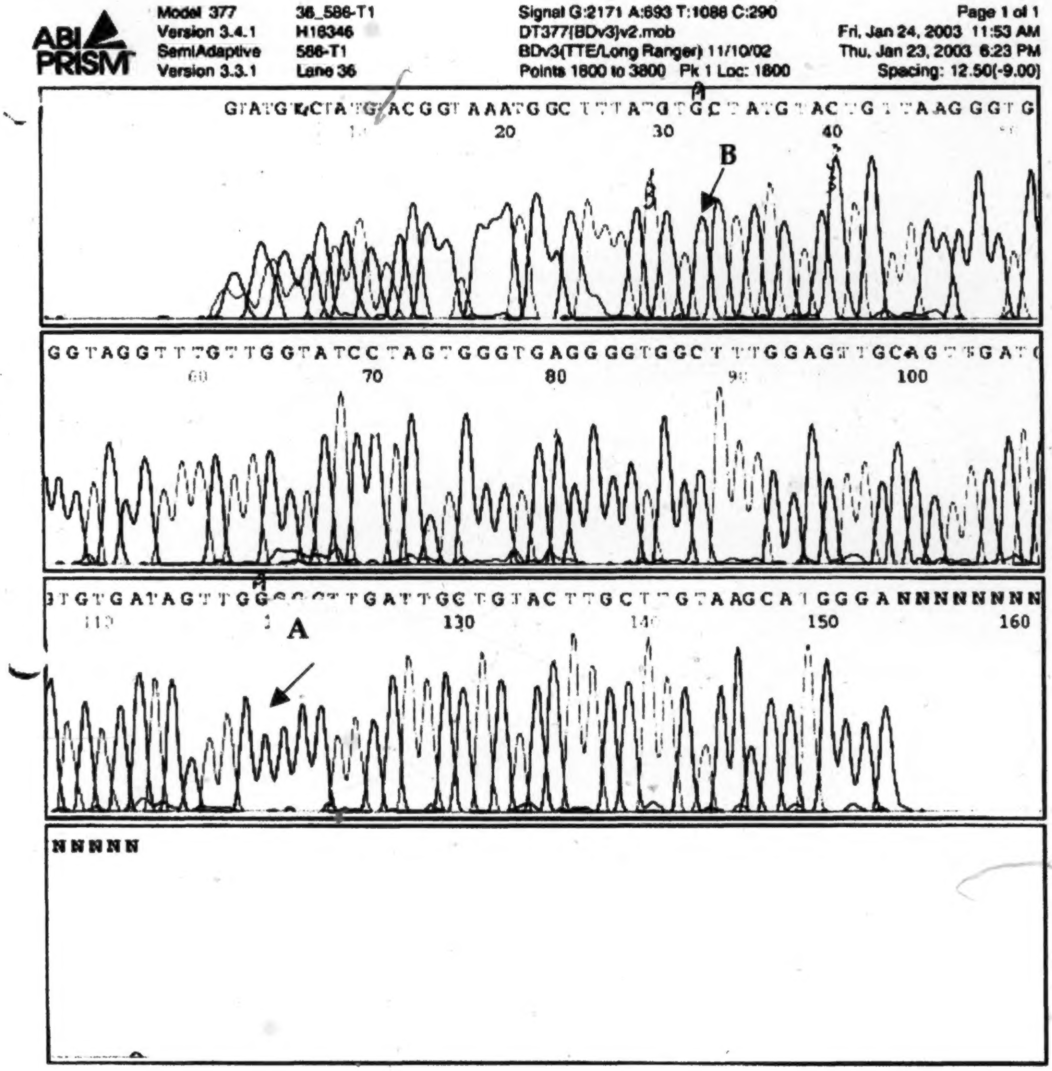
38_280-T2
H16346
280-T2
Lane 38

Signal G:1649 A:539 T:799 C:225
DT377{BDv3}v2.mob
BDv3{TTE/Long Ranger} 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Fri, Jan 24, 2003 11:53 AM
Thu, Jan 23, 2003 6:23 PM
Spacing: 12.50{-9.00}

A	16224	C/T
B	16311	C/T
C	16320	C/T

Extract 586 F1: H strand



Model 377
Version 3.4.1
SemiAdaptive
Version 3.3.1

36_586-T1
H16346
586-T1
Lane 36

Signal G:2171 A:693 T:1088 C:290
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Fri, Jan 24, 2003 11:53 AM
Thu, Jan 23, 2003 6:23 PM
Spacing: 12.50(-9.00)

A	16224	C
B	16311	T

Appendix 3: Primers for RFLP, HVI and amelogenin targets analysed in this study

Haplogroup	**RFLP**	**Primer**	**Sequence**	**Length (bp)**	**Anneal Temperature**	**Reference**
A	+HaeIII 663	L635 H708	5'–TGAAAATGTTTAGACGGCCTCACATG 5'–TAGAGGGTGAACTCACTGGAAC	108	58 °C	Handt *et al.*, 1996
B	COII/tRNA^{-Lys} 9 bp deletion	L8196 H8297	5'–ACAGTTTCATGCCCATCGTC 5'–ATGCTAAGTTAGCTTTACAG	112/121	58 °C	Handt *et al.*,1996
C	–HincII 13259	L13257 H13393	5'–AATCGTAGCCTTCTCCACTTCA 5'–TCCTATTTTTCGAATATCTTGTTC	180	58 °C	Handt *et al.*,1996
D	–AluI 5176	L5127 H5189	5'–ACTACCGCATTCTACTACTCA 5'–GGGTGGATGGAATTAAGGGTGT	106	54 °C	Handt *et al.*,1996
F	–HincII 12406	L12368 H12473	5'–CCCTGACTTCCCTAATTCCC 5'–TGTTGTGGGGAAGAGACTGA	125	56 °C	this study
G2a	–CfoI 7598	L7495 H7615	5'–TGATAGGGGAAGTAGCGTCTT 5'–ATGGCCTCCATGACTTTTTC	140	54°C	this study
M	+AluI 10397/ +DdeI 10394	L10361 H10458	5'–TCTGGCCTATGAGTGACTACAA 5'–TGAGGGGCATTTGGTAAATATG	120	56 °C	this study
X	–DdeI 1715	L1688 H1752	5'–AACTTAACTTGACCGCTCT 5'–TGCGCCAGGTTTCAATTTCTA	107	54°C	this study
HVI	n/a	H16346 L16211	5'–CCCATGCTTACAAGCAAGTA 5'–CAGTTTAGGGAAGAGCAGGG	176	54 °C	this study
Amelogenin	n/a	Amel1 Amel2	5'–CCCTGGGCTCTGTAAAGAATAGTG 5'–ATCAGAGCTTAAACTGGGAAGCTG	106/112	60 °C	Mannucci *et al.*, 1994

Appendix 4: mtDNA haplogroup frequencies for modern and Neolithic Asian populations analysed in this study.

*The polymorphisms defining the haplogroup "Other" lineages are described in the text

[a] Ancient Groups examined in this study

			mtDNA Haplogroups									
Group Name	Symbol	N	A	B	C	D	G2a	F	Other Asian	Other European	Gene Diversity (h)	Reference
Lokomotiv[a]	LM	31	0.13	0.00	0.03	0.23	0.03	0.49	0.00	0.10	0.71	This study
Ust-Ida[a]	UI	39	0.26	0.00	0.28	0.05	0.10	0.08	0.08	0.15	0.84	This study
Eygin Gol[a]	EG	46	0.17	0.02	0.130	0.37	0.02	0.09	0.09	0.11	n/a	Keyser–Tracqui *et al.*, 2003
Korea	KR	101	0.13	0.14	0.04	0.33	0.03	0.08	0.19	0.07	0.76	Snall *et al.*, 2002
Tibetian	TB	54	0.11	0.06	0.04	0.17	0.07	0.15	0.06	0.35	0.73	Torroni *et al.*, 1994
Mongolian	MG	42	0.05	0.02	0.12	0.17	0.00	0.07	0.26	0.36	0.61	Merriwether *et al.*, 1996
Mansi	MN	98	0.03	0.00	0.17	0.08	0.00	0.01	0.07	0.56	0.55	Derbeneva *et al.*, 2002a
Siberians	SB	411	0.26	0.00	0.33	0.16	0.00	0.00	0.24	0.00	0.73	Torroni *et al.*, 1993
Kets	KT	38	0.08	0.00	0.16	0.03	0.00	0.24	0.03	0.47	0.68	Derbeneva *et al.*, 2002b
Tofalar	TF	58	0.05	0.03	0.62	0.00	0.02	0.00	0.07	0.21	0.52	Derenko *et al.*, 2002a
Todjins	TD	48	0.04	0.08	0.48	0.04	0.00	0.02	0.18	0.15	0.61	Derenko *et al.*, 2002a
Evenks	EV	79	0.04	0.05	0.48	0.27	0.02	0.01	0.05	0.08	0.52	Derenko *et al.*, 2002a
Yakuts	YK	62	0.00	0.05	0.42	0.27	0.02	0.02	0.09	0.14	0.62	Derenko *et al.*, 2002a
Altai	AT	110	0.00	0.04	0.19	0.15	0.01	0.08	0.13	0.40	0.89	Derenko *et al.*, 2002a
Altai–Kizhi	AK	92	0.03	0.03	0.30	0.01	0.01	0.05	0.14	0.32	0.87	Derenko *et al.*, 2002b
Shorians	SH	42	0.00	0.02	0.07	0.01	0.00	0.43	0.02	0.36	0.76	Derenko *et al.*, 2002b
Khakassians	KK	54	0.04	0.06	0.35	0.09	0.00	0.22	0.00	0.24	0.81	Derenko *et al.*, 2002b
Sojots	SJ	34	0.09	0.03	0.18	0.50	0.09	0.00	0.00	0.12	0.72	Derenko *et al.*, 2002b
Tuvnians	TV	90	0.11	0.08	0.48	0.18	0.02	0.02	0.05	0.16	0.733	Derenko *et al.*, 2002a
Buryats	BT	91	0.02	0.07	0.29	0.33	0.14	0.010	0.04	0.10	0.79	Derenko *et al.*, 2002a

Appendix 5a: Summarised RFLP and HVI sequence data

Summarised mtDNA HVI and RFLP data for Kitoi and Serovo-Glazkovo

Key to characteristic polymorphisms:

A= HaeIII +663 D = AluI –5176
B=9bp del F= HincII –12406
C= HincII –16259 G2a = CfoI –7598
Other = DdeI/AluI –10394/–10397, HaeIII –663, HincII +12406

HVI variants suffixed with an asterix were not reported
*n/o = not obtained

Kitoi

Sample #	Burial #	Burial Type	Crania Status	Cemetery Sector	Sex	Age (y)	Uncal BP	DdeI 10394	AluI 10397	HaeIII 663	HincII 12406	HincII 13259	AluI 5176	9bp del	CfoI 7598	DdeI 1715	HVI Variants +16000	Hap
95–096a	2–1–1	M	A	2	Amb	20–25	6750		neg	neg	neg	pos	pos	neg			n/o	F
95–096b									neg	neg	neg	pos	pos	neg			n/o	
95–097	2–3–1	M	A	2	F	25–35		neg	neg	neg	neg					pos	n/o	F
95–097b								neg	neg	neg	neg					pos	n/o	
95–099a	4–1–1	S	P	2	M	25–35	6550		pos	neg		pos	neg	neg			n/o	D
95–099b									pos	neg		pos	neg	neg			n/o	
95–100a	6–1–1	S	P	2	M	Mature	6490		neg	neg	neg	pos	pos	neg			n/o	F
95–100b									neg	neg	neg	pos	pos	neg			n/o	
95–103a	10–2–1	M	A	2	M	20–25	6890	neg	neg	neg	pos					pos	231 256 270*	Other
95–103b								neg	neg	neg	pos					pos	n/o	
95–104a	10–3–1	M	A	2	M	50+	6770		neg	neg	neg	pos	pos				232A 249 304 311	F
95–104b									neg	neg	neg	pos	pos				232A 249 304 311	
95–106a	12–1–1	S	A	2	M	20–25	6640		neg	pos		pos	pos				223 290 319	A
95–105b									neg	pos		pos	pos				223 290 319	
95–107a	13–1–1	S	P	2	M	25–35	6200		pos	neg		pos	neg				223 270*	D
95–107b									pos	neg		pos	neg				n/o	
95–108a	14–3–1	M	P	2	M	7.5–9.5	6820	pos	neg	neg	pos	pos		neg			256 270	U5a
95–108b								pos	neg	neg	pos	pos		neg			256 270	
95–109a	14–4–4	M	P	2	M	15–20	6620		neg		neg	pos		neg			n/o	F
95–109b									neg		neg	pos		neg			232A 249 304 311*	
95–110a	15–1–1	S	P	2	M	50–60	6480		pos			pos	neg	neg			n/o	D
95–110b									pos			pos	neg	neg			n/o	
95–111a	16–1–1	S	P	2	M	45–55	6680		pos			pos	neg	neg			n/o	D
95–111b									pos			pos	neg	neg			n/o	

Kitoi (continued)

Sample #	Burial #	Burial Type	Crania Status	Cemetery Sector	Sex	Age (y)	UncalBP	DdeI 10394	AluI 10397	HaeIII 663	HincII 12406	HincII 13259	AluI 5127	9bp del	CfoI 7598	DdeI 1715	HVI Variants +16000	Hap
95–112a	17–1–1	S	A	2	M	45–55	6480		neg	pos		pos	pos	neg			223 290 319*	A
95–112b									neg	pos		pos	pos	neg			n/o	
95–113a	18–1–3	S	P	2	imm	6.5–7.5	6520		neg	neg	pos		pos				232A 249 304 311 *	F
95–113b									neg	neg	pos		pos				n/o	
95–114a	22–1–5	M	A	2	M	Mature	6660		pos	neg		neg		neg			223 298 327	C
95–114b									pos	neg		neg		neg			223 298 327	
95–115a	24–1–1	M	A	2	M	11.5–15	6660		neg	neg	neg						n/o	F
95–115b									neg	neg	neg						n/o	
95–116a	24–2–1	M	A	2	M	40–45	6620		neg	neg	neg	pos	pos				232A 249 304 311 *	F
95–116b									neg	neg	neg	pos	pos				n/o	
95–118a	27–1–1	S	P	4	M	15–18	6790		pos	neg		pos	neg				223	D
95–118b									pos	neg		pos	neg				223	
95–119a	28–1–1	S	P	4	F	35–40	6380	neg	neg	neg	pos			neg		pos	256 270	U5a
95–119b								neg	neg	neg	pos			neg		pos	256 270	
95–120a	29–1–1	S	P	4	M	25–30	6780		neg		neg	pos					232A 249 304 311 *	F
95–120b									neg		neg	pos					n/o	
95–121a	30–1–1	D	P	4	M	35–40	6800		neg		neg	pos					n/o	F
95–121b									neg		neg	pos					n/o	
95–122a	31–2–1	D	P	4	M	25–30	6900		neg	pos		pos	pos	neg			223 290 319	A
95–122b									neg	pos		pos	pos	neg			223 290 319	
95–124a	36–1–1	S	P	5	F	20–25	6600		neg	neg	neg		pos				n/o	F
95–124b									neg	neg	neg		pos				n/o	
95–125a	37–1–1	S	A	5	F	25–29	6700		neg	neg	neg			neg			232A 249 304 311 *	F
95–125b									neg	neg	neg			neg			n/o	
95–126a	38–1–1	D	P	6	Amb	50+	6700		neg	neg	neg	pos	pos	neg			n/o	F
95–126b									neg	neg	neg	pos	pos	neg			n/o	
95–127a	38–2–1	D	P	6	F	35–45	6720		neg	pos	pos	pos	pos				223 290 *	A
95–127b									neg	pos	pos	pos	pos				n/o	
95–128a	39–1–1	S	P	6	F	20–25	6720		pos			pos	neg				223 259*	D
95–128b									pos			pos	neg				n/o	
95–129a	41–1–1	T	P	7	F	15–20	6820		pos			pos	neg				223	D

Kitoi (continued)

Sample #	Burial #	Burial Type	Crania Status	Cemetery Sector	Sex	Age (y)	UncalBP	DdeI 10394	AluI 10397	HaeIII 663	HincII 12406	HincII 13259	AluI 5176	9bp del	CfoI 7598	DdeI 1715	HVI Variants -16000	Hap
95–129b									pos			pos	neg				223	
95–130a	42–1–1	S	P	7	M	40–50	7140		pos			pos	pos		neg		223 227 278	G2a
95–130b									pos			pos	pos		neg		223 227 278	
95–131a	44–1–1	D	P	7	M	35–39	6800		neg	neg	neg	pos	pos	neg	pos		n/o	F
95–131b									neg	neg	neg	pos	pos	neg	pos		n/o	
95–132a	44–2–1	D	P	7	M	30–39	6740		neg	neg	neg			neg			232A 249 304 311	F
95–132b									neg	neg	neg			neg			232A 249 304 311	

Serovo-Glazkovo

Sample #	Burial #	Burial Type	Crania Status	Cemetery Sector	Sex	Age (y)	UncalBP	DdeI 10394	AluI 10397	HaeIII 663	HincII 12406	HincII 13259	AluI 5127	9bp del	CfoI 7598	DdeI 1715	HVI Variants +16000	Hap
95–141a	5–1–1	S	Ser	D	M	7–9	4690		pos	neg	pos	pos	pos	neg	pos	pos	223	Other
95–141b									pos	neg	pos	pos	pos	neg	pos		223	
95–142a	9–1–1	S	Ser	D	M	0–6	4720	neg	neg	pos	pos	pos					n/o	A
95–142b									neg	pos	pos	pos					n/o	
95–143a	11–1–1	S	Ser	U	F	35–50	4870		pos	neg		pos	pos		neg		223 227 262 278	G2a
95–143b									pos	neg		pos	pos		neg		223 227 262 278	
95–144a	12–1–1	S	Glaz	D	F	50+	4220		pos			pos	neg				223 319	D
95–144b									pos			pos	neg				223 319	
95–145a	14–1–1	D	Ser	D	M	15–20	4750		neg	neg	neg			neg			n/o	F
95–145b									neg	neg	neg			neg			n/o	
95–146a	16–1–1	D	Ser	D	M	45+	4710		neg	neg	neg	pos	pos	neg			n/o	F
95–146b									neg	neg	neg	pos	pos	neg			n/o	
95–147a	16–2–1	D	Ser	D	M	50+	4930		neg	neg		pos	pos				311	Other
95–147b									neg	neg		pos	pos				311	
95–148a	18–1–1	S	Ser	D	F	11–13	4690	neg	neg	neg	pos					pos	256 270	U5a
95–148b								neg	neg	neg	pos					pos	256 270	
95–149a	19–1–1	S	Glaz	D	M	30–35	4270		pos			neg	pos				n/o	C
95–149b									pos			neg	pos				n/o	
95–150a	20–1–1	D	Ser	U	M	18–24	4840		pos			neg	pos				223 298	C
95–150b									pos			neg	pos				223 298	
95–151a	20–2–1	D	Ser	U	F	18–25	4840		pos	neg	pos	pos	pos		pos		223 227 262 278*	Other
95–151b									pos	neg	pos	pos	pos		pos		n/o	
95–152a	22–1–1	S	Ser	U	F	15–20	4880		pos	neg	pos		pos		pos	pos	223 227 262 278	Other
95–152b									pos	neg	pos		pos		pos	pos	223 227 262 278	
95–153a	24–1–1	S	Glaz	U	F	14–18	4200		pos	neg		neg	pos				223 298 327	C
95–153b									pos	neg		neg	pos				223 298 327	
95–154a	26–1–1	M	Ser	D	M	13–15	4740		neg	pos	pos	pos					223 227 290 319	A
95–154b									neg	pos	pos	pos					223 227 290 319	
95–155a	26–4–1	M	Ser	D	M	10–12	4800	neg	neg	pos	pos		pos				223 227 290 319	A
95–155b								neg	neg	pos	pos		pos				223 227 290 319	
95–156a	26–5–1	M	Ser	D	F	5–7			neg	neg	neg	pos					n/o	F
95–156b									neg	neg	neg	pos					n/o	
95–157a	29–1–1	S	Glaz	U	M	45–60	3870		neg	pos	pos						223 290 319	A
95–157b									neg	pos	pos						223 290 319	
95–158a	30–1–1	S	Ser	D	F	40–60	4670		neg	pos	pos	pos	pos				n/o	A
95–158b									neg	pos	pos	pos	pos				223 290 319 *	
95–159a	31–1–1	S	Ser	D	M	9–11	4670	neg	neg	neg	pos	pos			pos	pos	223 290 292 c/t*	Other
95–159b								neg	neg	neg	pos	pos			pos	pos	223 290 292 c/t*	
95–161a	33–1–1	D	Ser	D	M	12–15	4890	neg	neg	neg	pos	pos	pos			pos	311	Other

Serovo-Glazkovo (continued)

Sample #	Burial #	Burial Type	Crania Status	Cemetery Sector	Sex	Age (y)	UncalBP	DdeI 10394	AluI 10397	HaeIII 663	HincII 12406	HincII 13259	AluI 5127	9bp del	CfoI 7598	DdeI 1715	HVI Variants +16000	Hap
95–161a								neg	neg	neg	pos	pos	pos			pos	311	
95–162a	33–2–1	D	Ser	D	M	13–16	4680	pos	pos	neg	pos	pos	pos		pos	pos	223 227 262 278	Other
95–162b								pos	pos	neg	pos	pos	pos		pos	pos	223 227 262 278	
95–163a	36–1–1	D	Ser	D	F	30–40	4630		pos			pos	neg				223 319	D
95–163b									pos			pos	neg				223 319	
95–164a	38–1–1	S	Ser	D	M	45–60	4730		pos			neg	pos				223 298 327	C
95–164b									pos			neg	pos				n/o	
95–165a	40–1–1	S	Glaz	D	F	25–35	3650		pos	neg	pos	neg	pos		pos	pos	n/o	C
95–165b									pos	neg	pos	neg	pos		pos	pos	n/o	
95–166a	41–1–1	S	Ser	D	M	50–60	4790		pos			pos	pos		neg		n/o	G2a
95–166b									pos			pos	pos		neg		n/o	
95–167a	42–1–1	S	Glaz	D	M	60+	4930		neg	pos	pos	pos	pos				223 290 319 *	A
95–167b									neg	pos	pos	pos	pos				n/o	
95–168a	43–1–1	D	Ser	D	M	Mature			pos	neg	pos	pos	pos		neg		n/o	G2a
95–168b									pos	neg	pos	pos	pos		neg		n/o	
95–169a	44–1–1	T	Ser	U	F	7–9y	4590		neg	pos	pos						223 290 319	A
95–169b									neg	pos	pos						223 290 319	
95–170a	44–2–1	T	Ser	U	F	4–5y	4630		neg	pos	pos						223 290 319	A
95–170b									neg	pos	pos						223 290 319	
95–171a	44–3–1	T	Ser	U	imm	9–11y	4890		neg	pos	pos						223 290 *	A
95–171b									neg	pos	pos						n/o	
95–172a	45–1–1	S	Glaz	D	M	22–30	3760		pos			neg	pos				223 298 327	C
95–172b									pos			neg	pos				223 298 327	
95–173a	47–1–1	S	Glaz	U	M	30–40	3770		pos	neg	pos	neg					n/o	C
95–173b									pos	neg	pos	neg					n/o	
95–174a	48–1–1	S	Glaz	U	M	50+	3830		pos			neg	pos				223 298 327*	C
95–174b									pos			neg	pos				223 298*	
95–175a	51–1–1	S	Glaz	U	M	Mature	3960		pos			neg	pos				223 298 327 *	C
95–175b									pos			neg	pos				n/o	
95–176a	52–1–1	D	Ser	U	F	60+	4850		neg	neg	neg	pos	pos				CRS*	Other
95–176b									neg	neg	neg	pos	pos				CRS*	
95–177a	53–2–1	D	Ser	U	imm	4–8y	4570		neg	pos	pos						n/o	A
95–177b									neg	pos	pos						n/o	
95–178a	54–1–1	S	Ser	U	M	60+	4710		pos			neg	pos				n/o	C
95–178b									pos			neg	pos				n/o	
95–179a	55–1–1	D	Ser	U	M	2–4y	4730		pos			neg	pos				223 288 298 327*	C
95–179b									pos			neg	pos				n/o	
95–180a	55–2–1	D	Ser	U	M	15–18	4580		pos	neg	pos	pos	pos		neg		n/o	G2a
95–180b									pos	neg	pos	pos	pos		neg		n/o	

Appendix 5b: Representative samples for RFLP analysis

Haplogroup A Amplification + RFLP digest (Handt et al., 1996)

Target:	108 bp fragment flanking *HaeIII* restriction site at np 663
Primers:	L635 5'–TGAAAATGTTTAGACGGCCTCACATG H708 5'–TAGAGGGTGAACTCACTGGAAC

Reaction Conditions

PE480/2400	40 cycles	94 °C	30s	56 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	56°C	90s	72 °C	60s	72 °C	5 min

*final extension step

Interpretation: haplogroup A= gain of restriction site

Key:		
	N	negative PCR control
	P	positive PCR control
	Haplogroup A positive	154a, b,; 155a, b
	Haplogroup A negative	97a, b; 123a, b; 141a, b
	no amplification	162a

N
P
97a
97b
123a
123b
141a
141b
152a
152b
154a
154b
155a
155b
162a
176a
176b

Haplogroup B Amplification

Target: 121 bp fragment flanking 9–bp deletion from np 8272–8281
(Handt *et al.*, 1996)

Primers:
L8196 5'–ACAGTTTCATGCCCATCGTC
H8297 5'–ATGCTAAGTTAGCTTTACAG

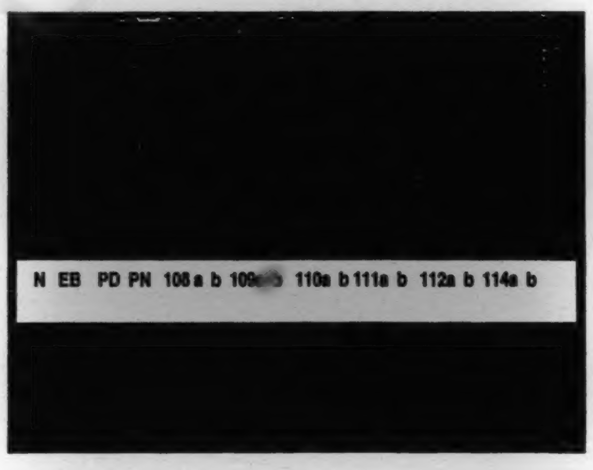
Reaction Conditions

PE480/2400	40 cycles	94 °C	30s	58 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	54°C	90s	72 °C	60s	72 °C	5 min

*final extension step

Interpretation: Haplogroup B = 9–bp deletion

Key		
	N	negative PCR control
	EB	extraction blank
	PD	positive PCR control with 9 bp deletion
	PN	positive PCR control without deletion
	Haplogroup B positive	none
	Haplogroup B negative	108a, b; 109a, b; 110b, 111a, b; 114a, b
	not interpretable	110a, 112a,b



Haplogroup C Amplification +RFLP digest (Handt et al., 1996)

Target: 180 bp region flanking *HincII* restriction site at np 13259

Primers:
L13257 5'–AATCGTAGCCTTCTCCACTTCA
H13393 5'–TCCTATTTTTCGAATATCTTGTTC

Reaction Conditions

PE480/2400	40 cycles	94 °C	30s	56 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	56°C	90s	72 °C	60s	72 °C	5 min

*final extension step

Interpretation: Haplogroup C= loss of restriction site

Key:

N	negative PCR control
EB	extraction blank
PC	positive PCR control, cut
PU	positive PCR control, uncut
Haplogroup C positive	172b; 175a,b; 178b; 179b
Haplogroup C negative	176a,b
not interpretable	172a; 178a; 179a

N
EB
PC
PU
172a b
175a b
176a b
178a b
179a b

Haplogroup D Amplification and RFLP digest (Handt *et al.*, 1996)

Target: 106 bp fragment flanking AluI restriction site at np 5176
Primers: L5127 5'–ACTACCGCATTCTACTACTCA
H5189 5'–GGGTGGATGGAATTAAGGGTGT

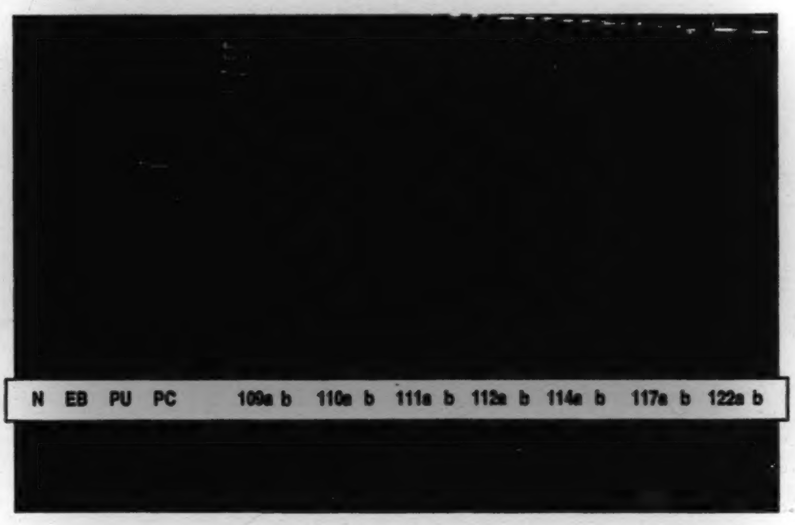
Reaction Conditions

PE480/2400	40 cycles	94 °C	30s	52 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	54°C	90s	72 °C	60s	72 °C	5 min

*final extension step

Interpretation: Haplogroup D = loss of restriction site

Key:	
N	negative PCR control
EB	extraction blank
PU	positive PCR control uncut
PC	positive PCR control cut
Haplogroup D positive	110b; 111a, b
Haplogroup D negative	109a, b; 112a,b; 114,b; 117a; 122a
not interpretable	110a, 117b, 122b



Haplogroup F amplification +RFLP digest

Target: 140 bp fragment flanking *HincII* restriction site at np 12406

Primers:

L12368	5'–CCCTGACTTCCCTAATTCCC
H12473	5'–TGTTGTGGGAAGAGACTGA

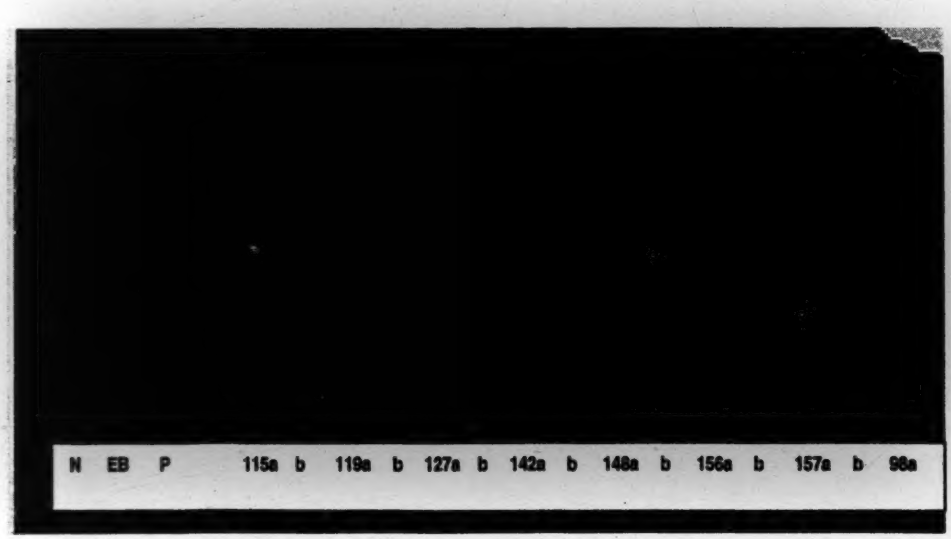
Reaction Conditions

PE480/2400	40 cycles	94 °C	30s	58 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	58°C	90s	72 °C	60s	72 °C	5 min*

*final extension step

Interpretation: Haplogroup F = loss of restriction site

Key:	
N	negative PCR control
EB	extraction blank
P	positive PCR control
Haplogroup F positive	115a,b 156a
Haplogroup F negative	119a,b; 127b; 142a,b; 148a; 157a,b; 98a
not interpretable	127a; 148b; 156b



Haplogroup G2a Amplification and RFLP digest

Target: 140 bp fragment flanking *CfoI* 7598 restriction site

Primers:	L7495	5′–TGATAGGGGAAGTAGCGTCTT
	H7615	5′–ATGGCCTCCATGACTTTTTC

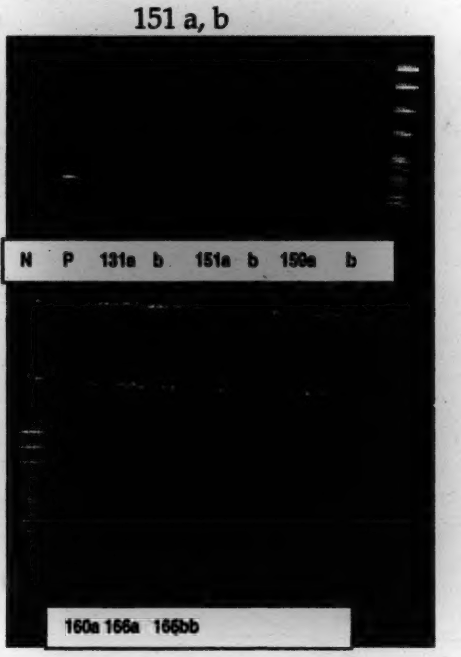
Reaction Conditions

PE480/2400	40 cycles	94 °C	30s	54 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	54°C	90s	72 °C	60s	72 °C	5 min

*final extension step

Interpretation: Haplogroup G2a= loss of restriction site

Key:	N	negative PCR control
	P	positive PCR control
	Haplogroup G2a positive	166 a, b
	Haplogroup G2a negative	131 a, b; 159a,b; 160a
	not interpretable	151 a, b



Haplogroup M Amplification and *AluI* RFLP digest

Target:	120 bp fragment flanking *AluI* 10397 restriction site	
Primers:	L10361	5'–TCTGGCCTATGAGTGACTACAA
	H10458	5'–TGAGGGGCATTTGGTAAATATG

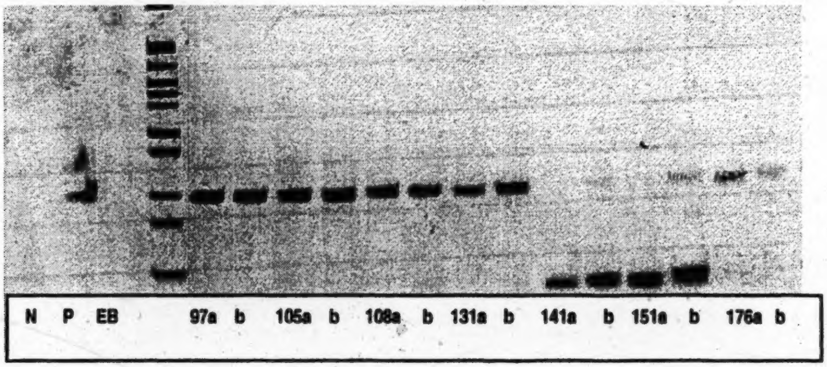
Reaction Conditions

PE480/2400	40 cycles	94 °C	30s	54 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	54°C	90s	72 °C	60s	72 °C	5 min

*final extension step

Interpretation: Haplogroup M = gain of site

Key:	N	negative PCR control
	P	positive PCR control
	Haplogroup M positive	141a,b; 151 a,b
	Haplogroup M negative	97a,b; 105a,b; 108a,b; 131a,b; 176a,b



Haplogroup M Amplification and *DdeI* RFLP digest

Target: 120 bp fragment flanking DdeI 10394 restriction site

Primers: same as for haplogroup M AluI 10397 amplification

Reaction Conditions

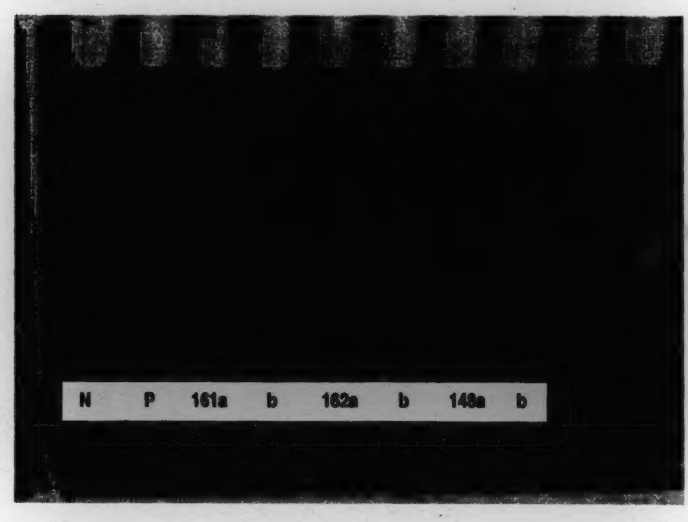
PE480/2400	40 cycles	94 °C	30s	54 °C	60s	72°C	30s	72 °C	5 min*
MJ PTC	40 cycles	95 °C	60s	54°C	90s	72 °C	60s	72 °C	5 min

*final extension step

Interpretation: Haplogroup M = gain of *AluI* 10397 and *DdeI* 10394 sites

Key:

N	negative PCR control
P	positive PCR control
Haplogroup M positive	162 a,b
Haplogroup M negative	161a,b; 148b
Not interpretable	148a



Haplogroup X Amplification and RFLP digest

Target: 107 bp fragment flanking *DdeI* restriction site at np 1715

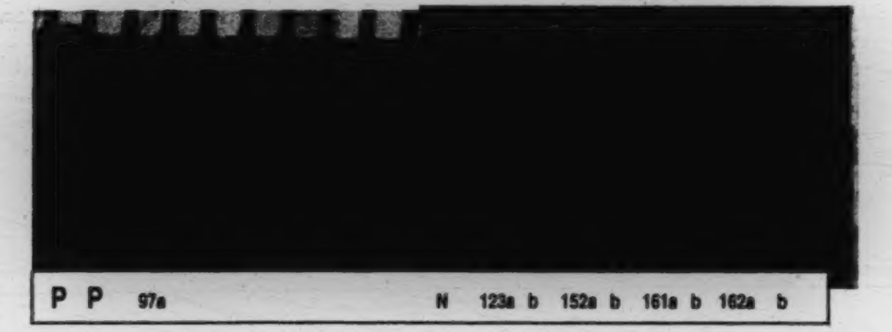
Primers:
H 1752 5′–AACTTAACTTGACCGCTCT
L 1688 5′–TGCGCCAGGTTTCAATTTCTA

Interpretation: Haplogroup X = loss of restriction site

Reaction Conditions

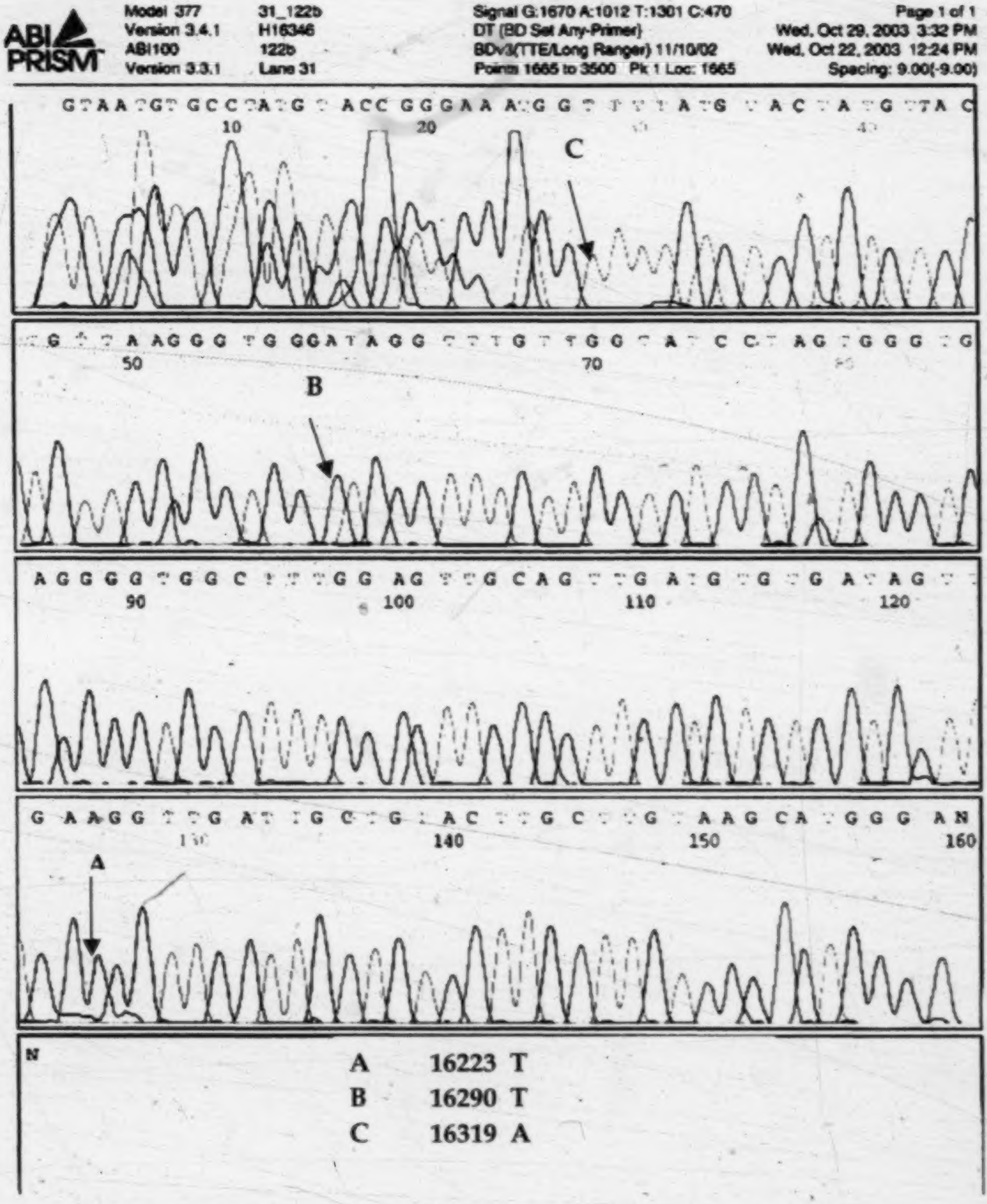
MJ PTC 40 cycles 95 °C 60s 58°C 90s 72 °C 60s 72 °C
5 min

Key	N	negative PCR control
	P	positive PCR control
	Haplogroup X positive	none
	Haplogroup X negative	97a, 123a,b; 152a,b; 161a,b; 162 a
	Not interpretable	162b



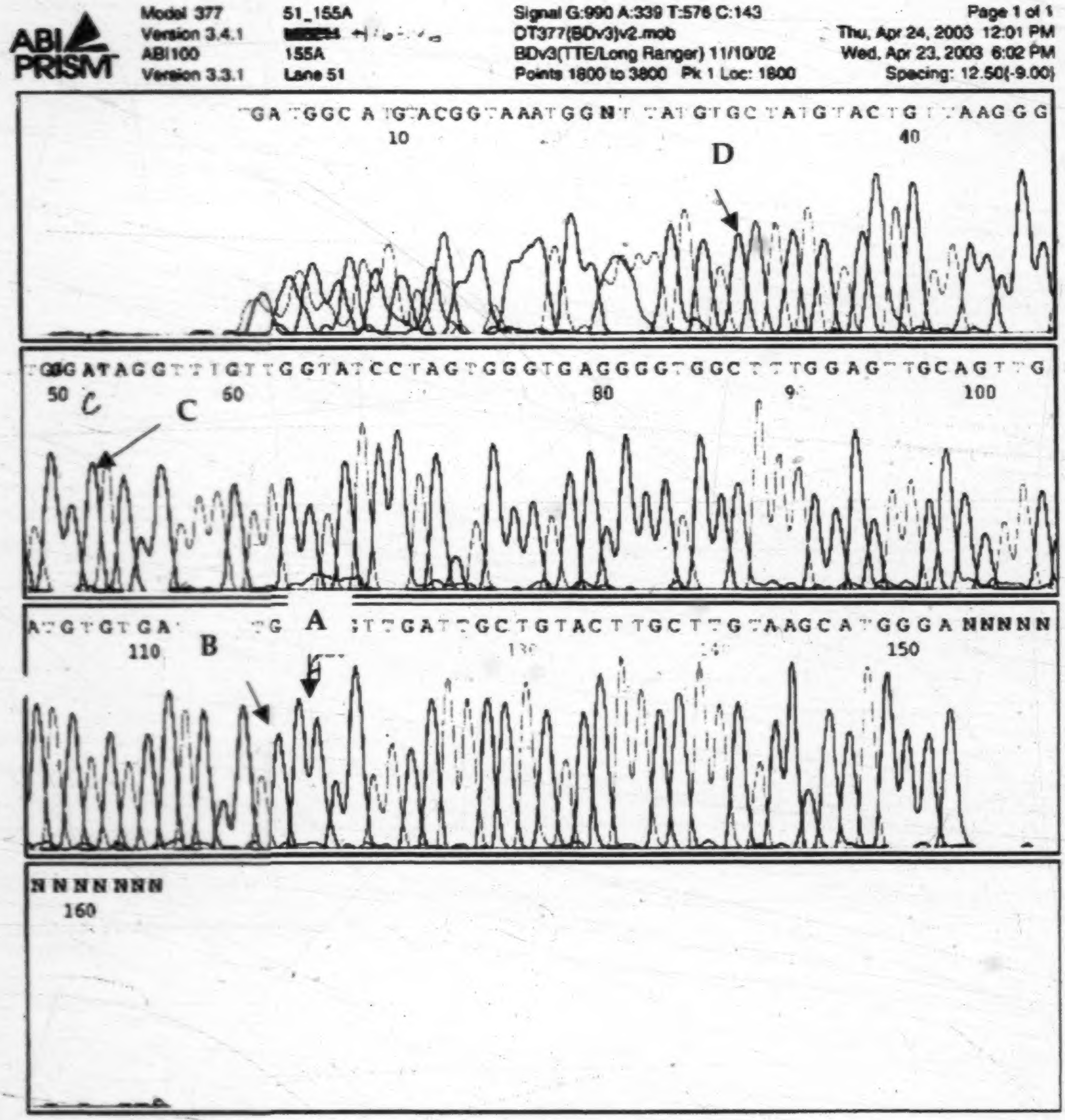
Appendix 5c: Representative samples of aDNA HVI sequences

95–122 (Lokomotiv): Haplogroup A variant 1 H strand



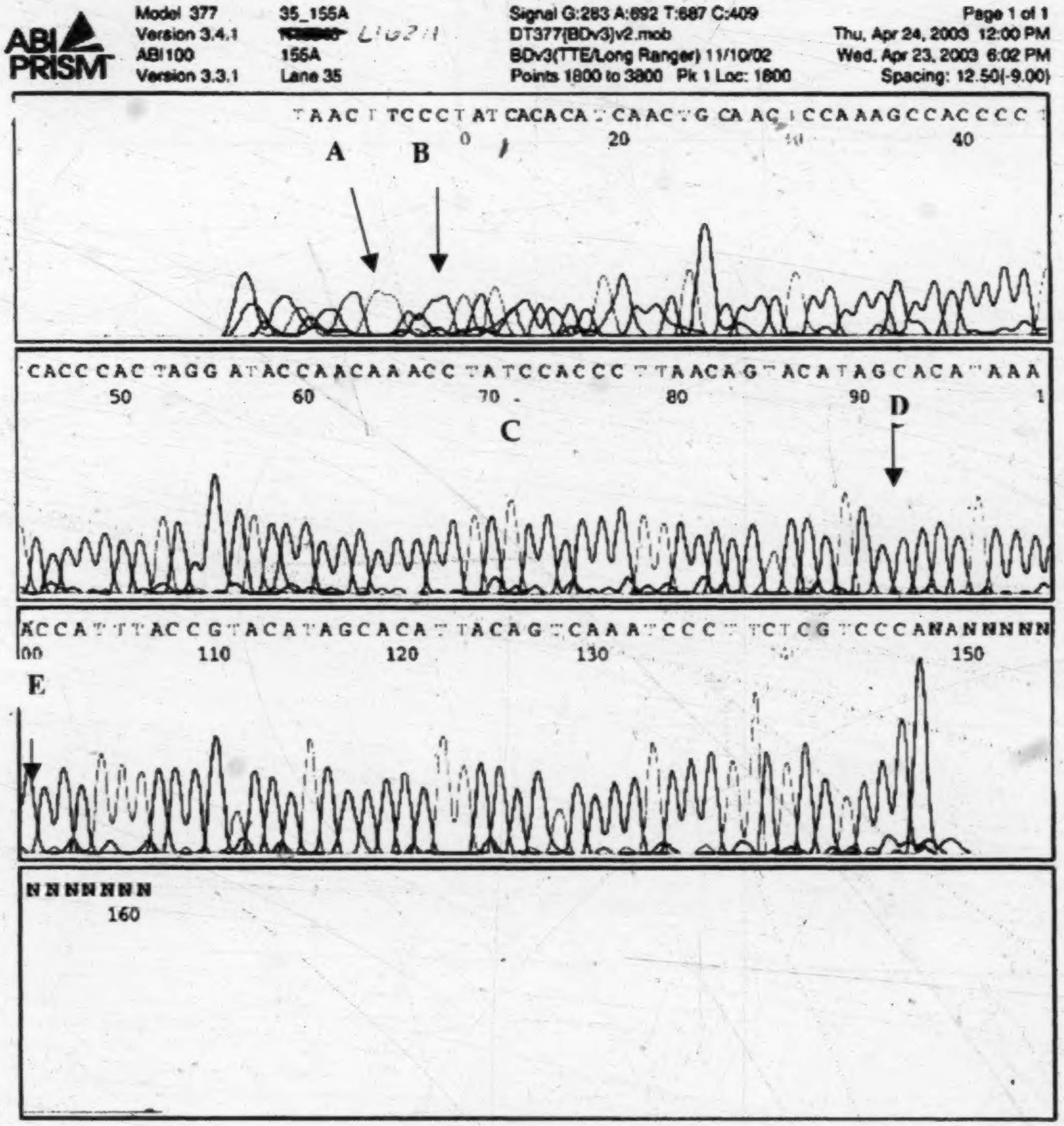
A	16223	T
B	16290	T
C	16319	A

95–155 (Ust-Ida) Haplogroup A variant 2 H strand



Key	A	16223	T
	B	16227	C
	C	16290	T
	D	16311	C

95–155 (Ust-Ida): Haplogroup A variant 2 L strand



ABI PRISM

Model 377
Version 3.4.1
ABI100
Version 3.3.1

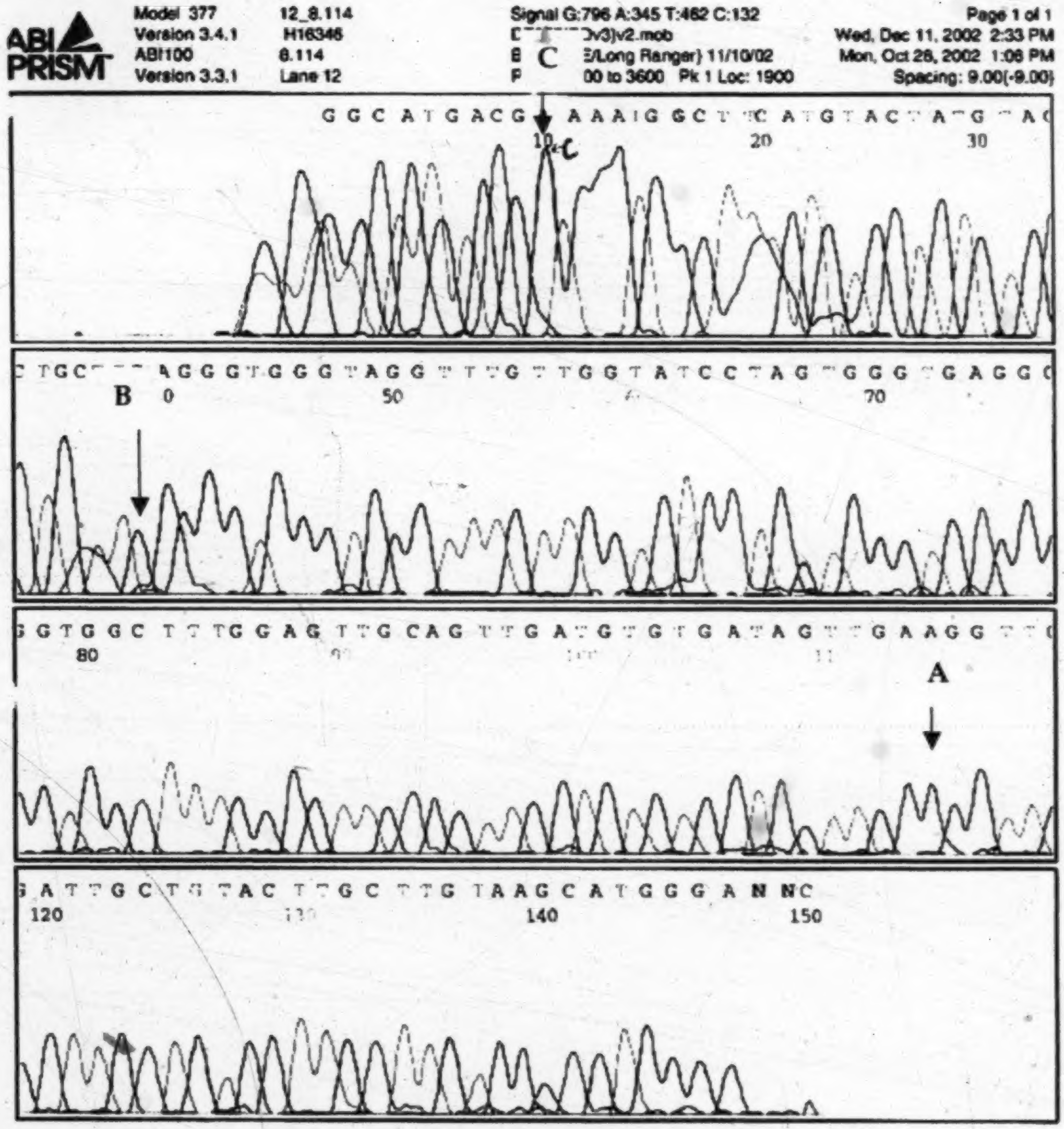
35_155A
[redacted] LiG2:1
155A
Lane 35

Signal G:283 A:692 T:687 C:409
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Thu, Apr 24, 2003 12:00 PM
Wed, Apr 23, 2003 6:02 PM
Spacing: 12.50(-9.00)

A	16223	T
B	16227	C
C	16290	T
B	16311	C
C	16319	A

95–114 (Lokomotiv): Haplogroup C H strand



A	**16223**	**T**
B	**16298**	**C**
C	**16327**	**T**

95–118 (Lokomotiv): Haplogroup D variant 1 H strand

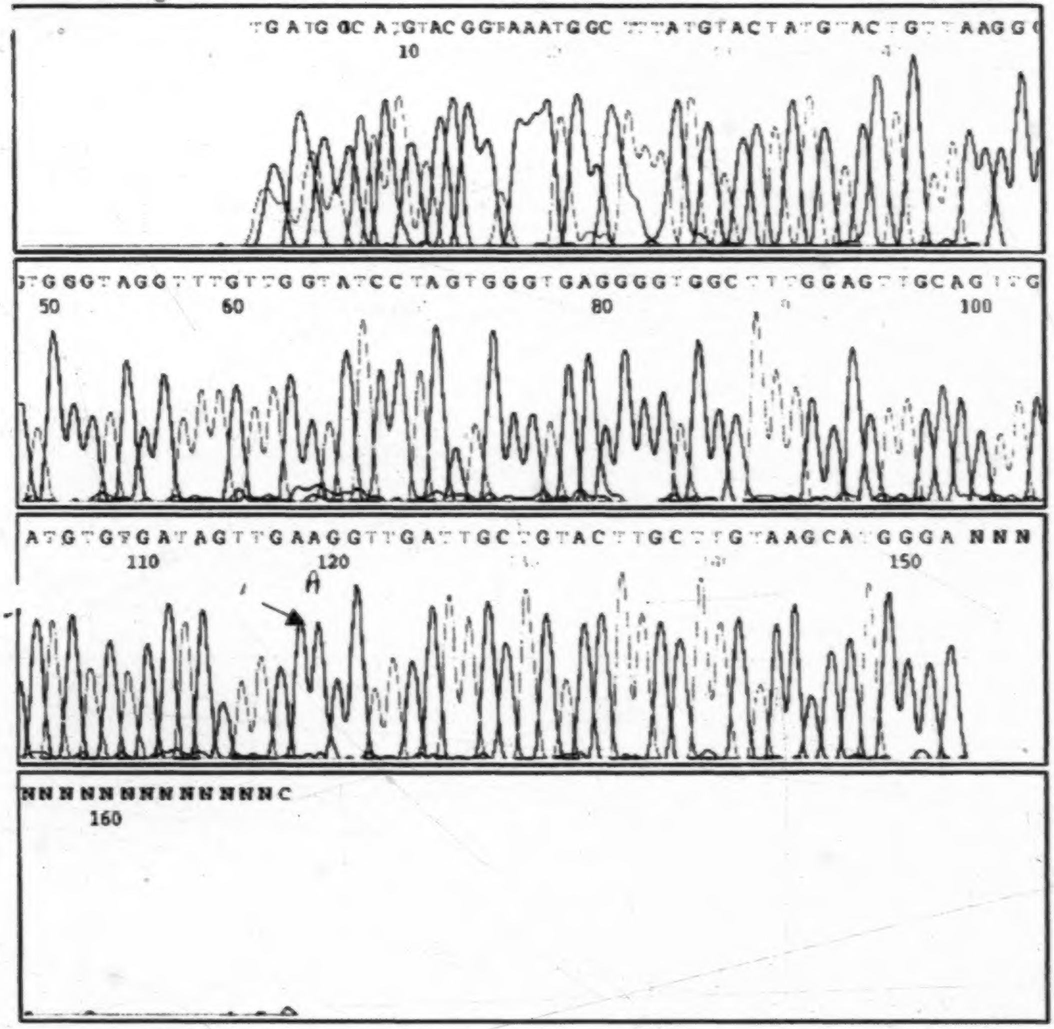
ABI PRISM

Model 377
Version 3.4.1
ABI100
Version 3.3.1

09_118A
H16346
118A
Lane 9

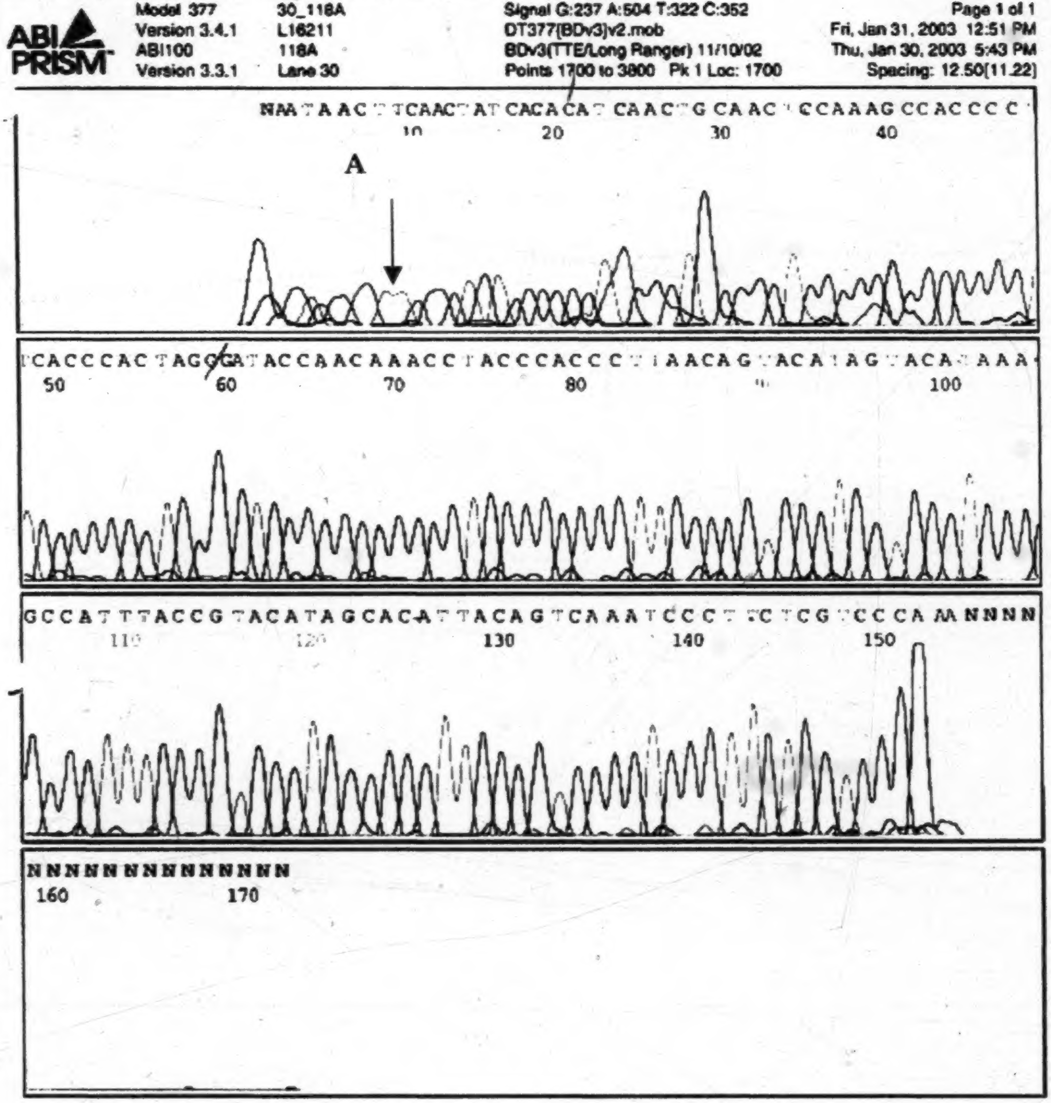
Signal G:1416 A:485 T:689 C:191
DT377(BDv3)v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1700 to 3800 Pk 1 Loc: 1700

Page 1 of 1
Fri, Jan 31, 2003 12:51 PM
Thu, Jan 30, 2003 5:43 PM
Spacing: 12.50(-9.00)



A 16223 T

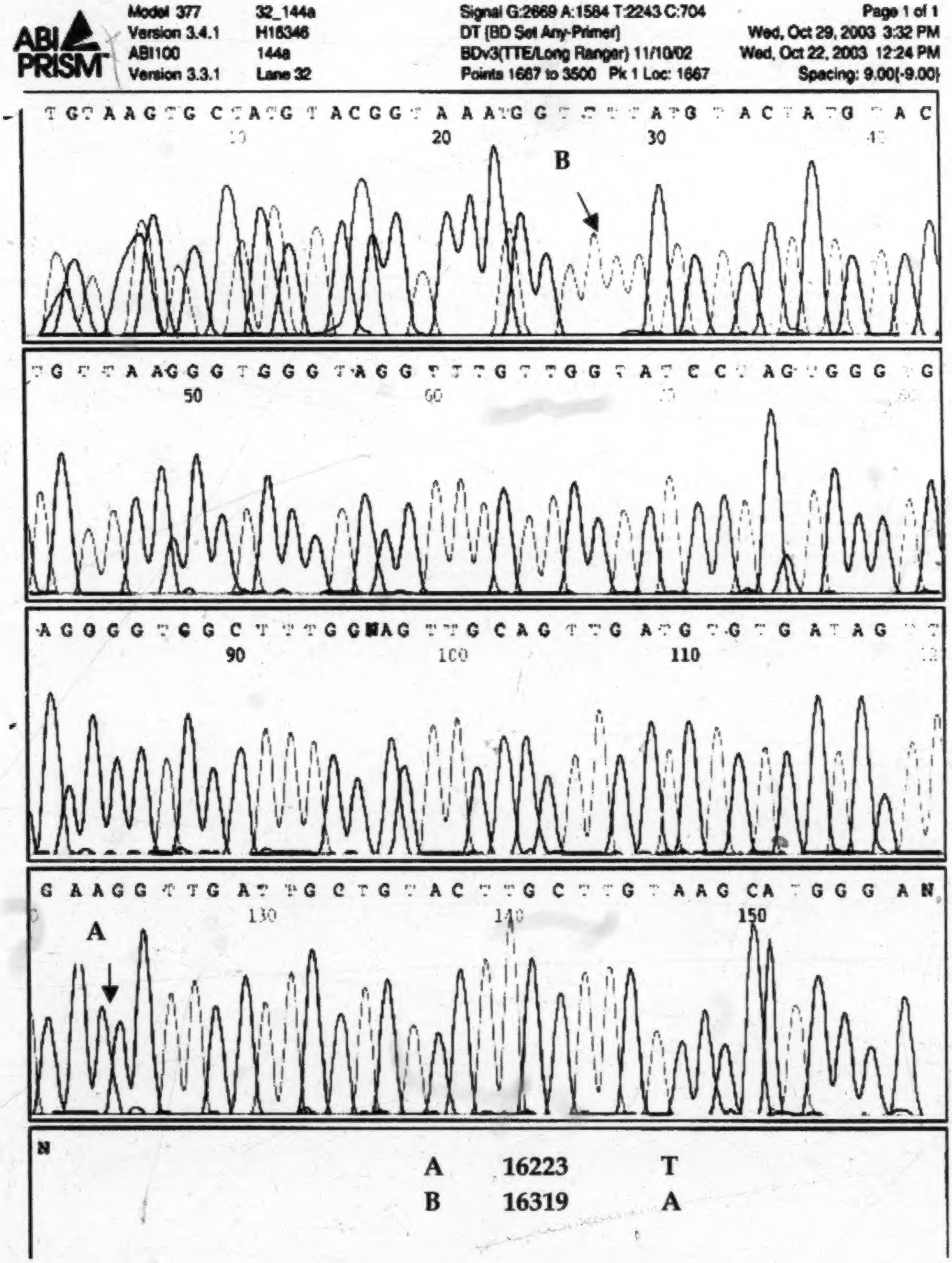
95–118 (Lokomotiv): Haplogroup D variant 1 H strand



ABI PRISM
Model 377
Version 3.4.1
ABI100
Version 3.3.1
30_118A
L16211
118A
Lane 30
Signal G:237 A:504 T:322 C:352
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1700 to 3800 Pk 1 Loc: 1700
Page 1 of 1
Fri, Jan 31, 2003 12:51 PM
Thu, Jan 30, 2003 5:43 PM
Spacing: 12.50[11.22]

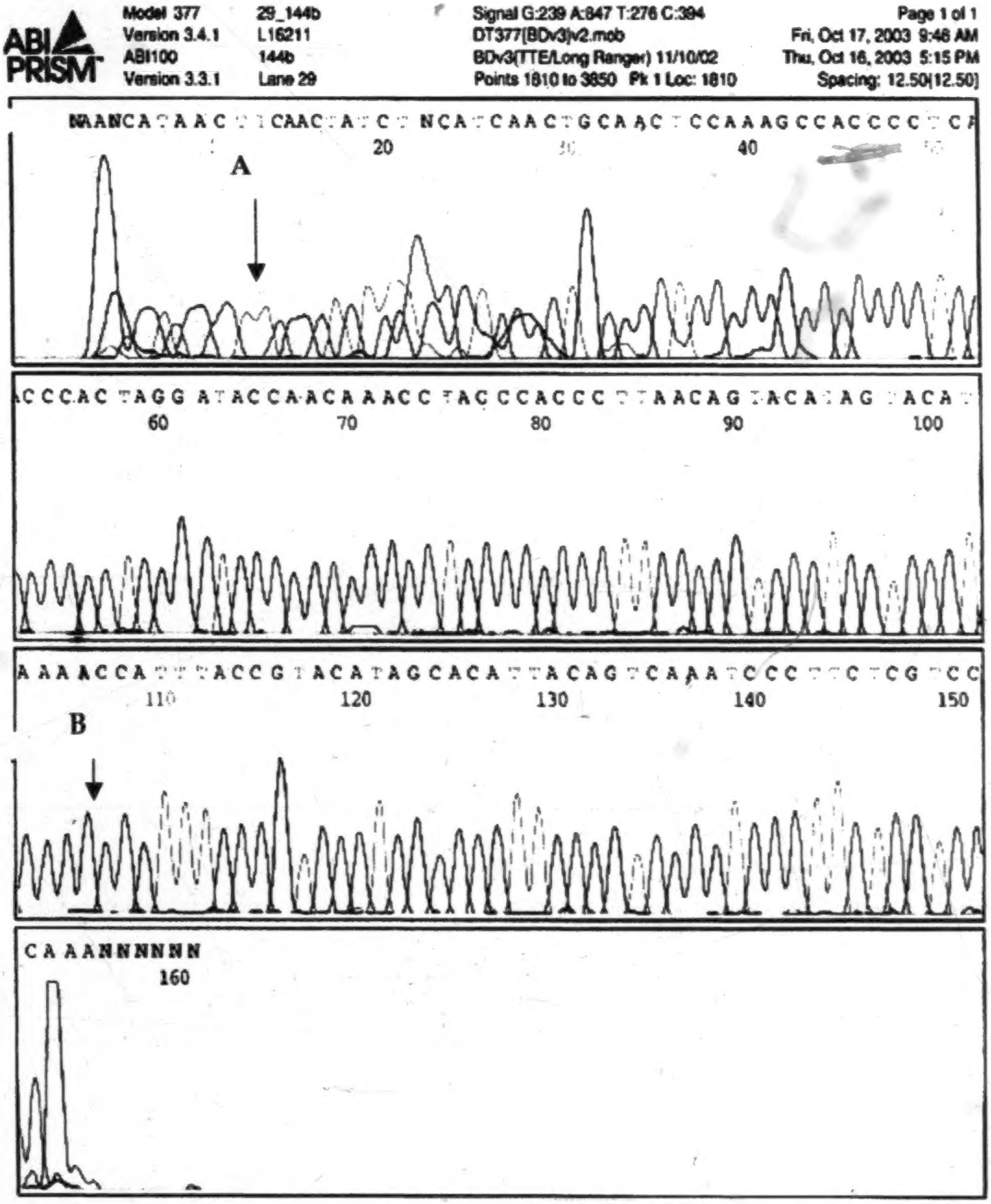
A **16223** **T**

95–144 (Ust-Ida): Haplogroup D variant 2: H strand



A 16223 T
B 16319 A

95–144 (Ust-Ida) Haplogroup D variant 2 L strand


ABI PRISM

Model 377 29_144b
Version 3.4.1 L16211
ABI100 144b
Version 3.3.1 Lane 29

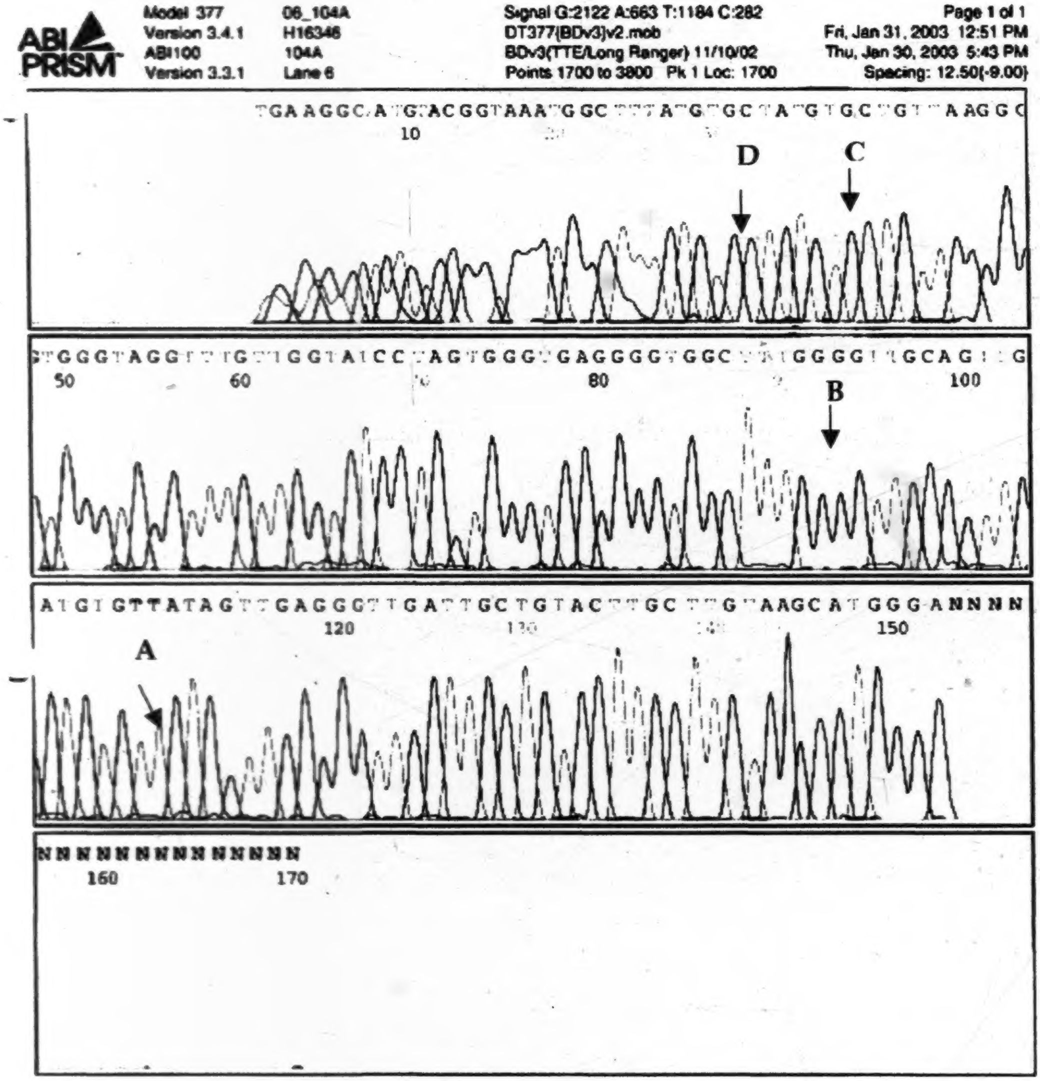
Signal G:239 A:847 T:276 C:394
DT377[BDv3]v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1810 to 3850 Pk 1 Loc: 1810

Page 1 of 1
Fri, Oct 17, 2003 9:46 AM
Thu, Oct 16, 2003 5:15 PM
Spacing: 12.50[12.50]

A	**16223**	**T**
B	**16319**	**A**

(Hap 1)

95–104 (Lokomotiv) Haplogroup F H strand



ABI PRISM

Model 377
Version 3.4.1
ABI100
Version 3.3.1

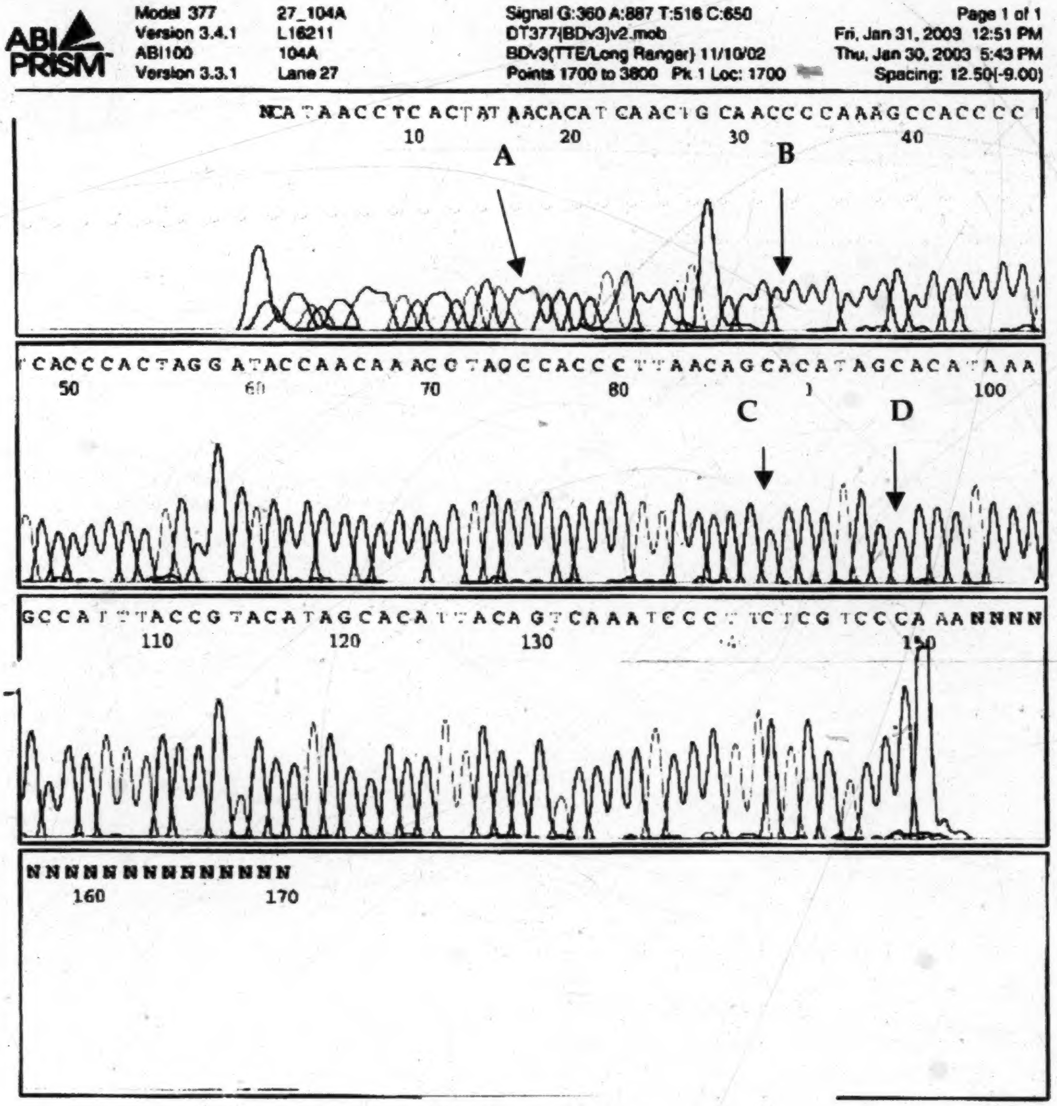
06_104A
H16346
104A
Lane 6

Signal G:2122 A:663 T:1184 C:282
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1700 to 3800 Pk 1 Loc: 1700

Page 1 of 1
Fri, Jan 31, 2003 12:51 PM
Thu, Jan 30, 2003 5:43 PM
Spacing: 12.50(-9.00)

A	16232	A
B	16249	C
C	16304	C
D	16311	C

95–104 (Lokomotiv): Haplogroup F L strand



Model 377
Version 3.4.1
ABI100
Version 3.3.1

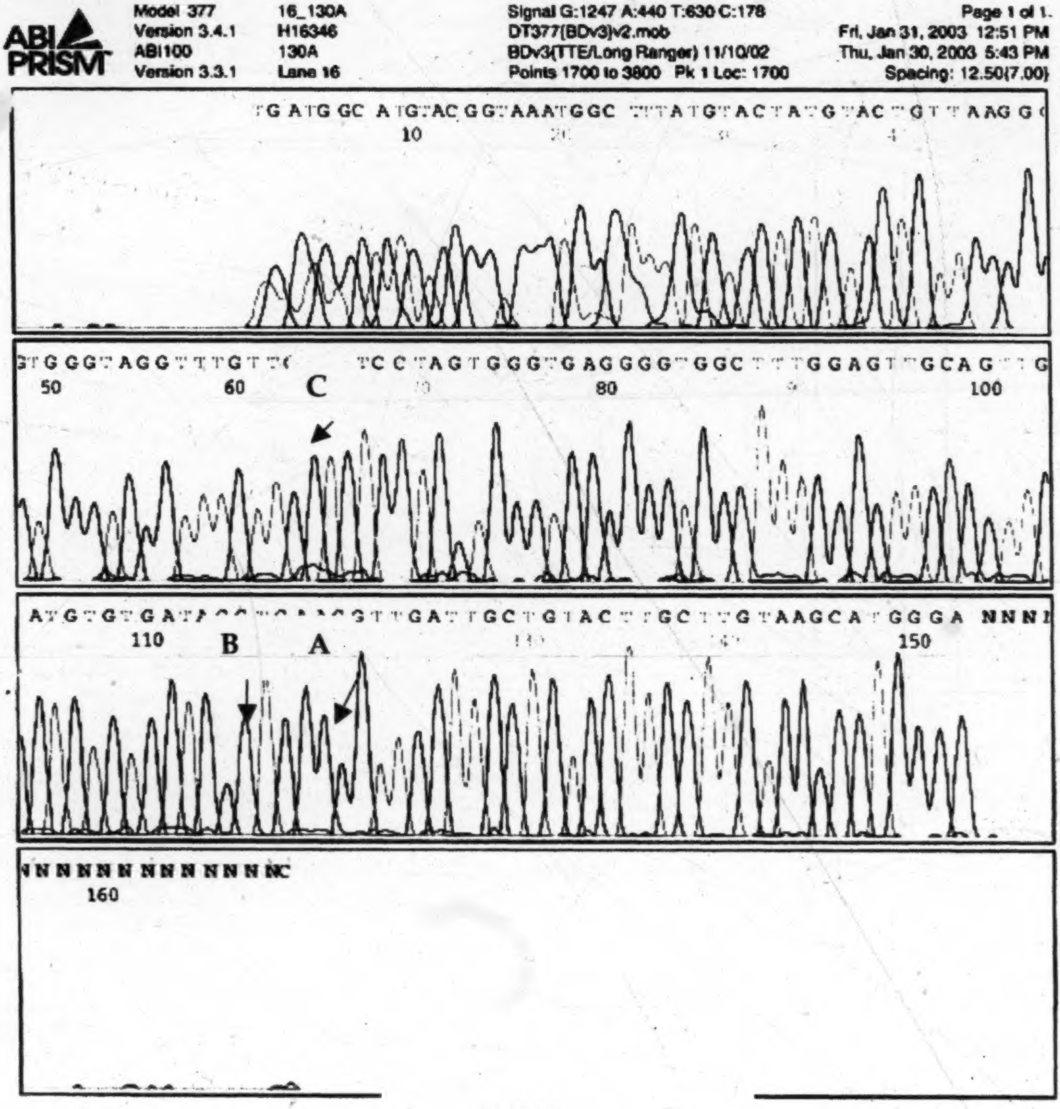
27_104A
L16211
104A
Lane 27

Signal G:360 A:887 T:516 C:650
DT377{BDv3}v2.mob
BDv3{TTE/Long Ranger} 11/10/02
Points 1700 to 3800 Pk 1 Loc: 1700

Page 1 of 1
Fri, Jan 31, 2003 12:51 PM
Thu, Jan 30, 2003 5:43 PM
Spacing: 12.50(-9.00)

A	16232	A
B	16249	C
C	16304	C
D	16311	C

95–130 (Lokomotiv): Haplogroup G2a variant 1 H strand



A	16223	T
B	16227	G
C	16278	T

95–130 (Lokomotiv): Haplogroup G2a variant 1 L strand

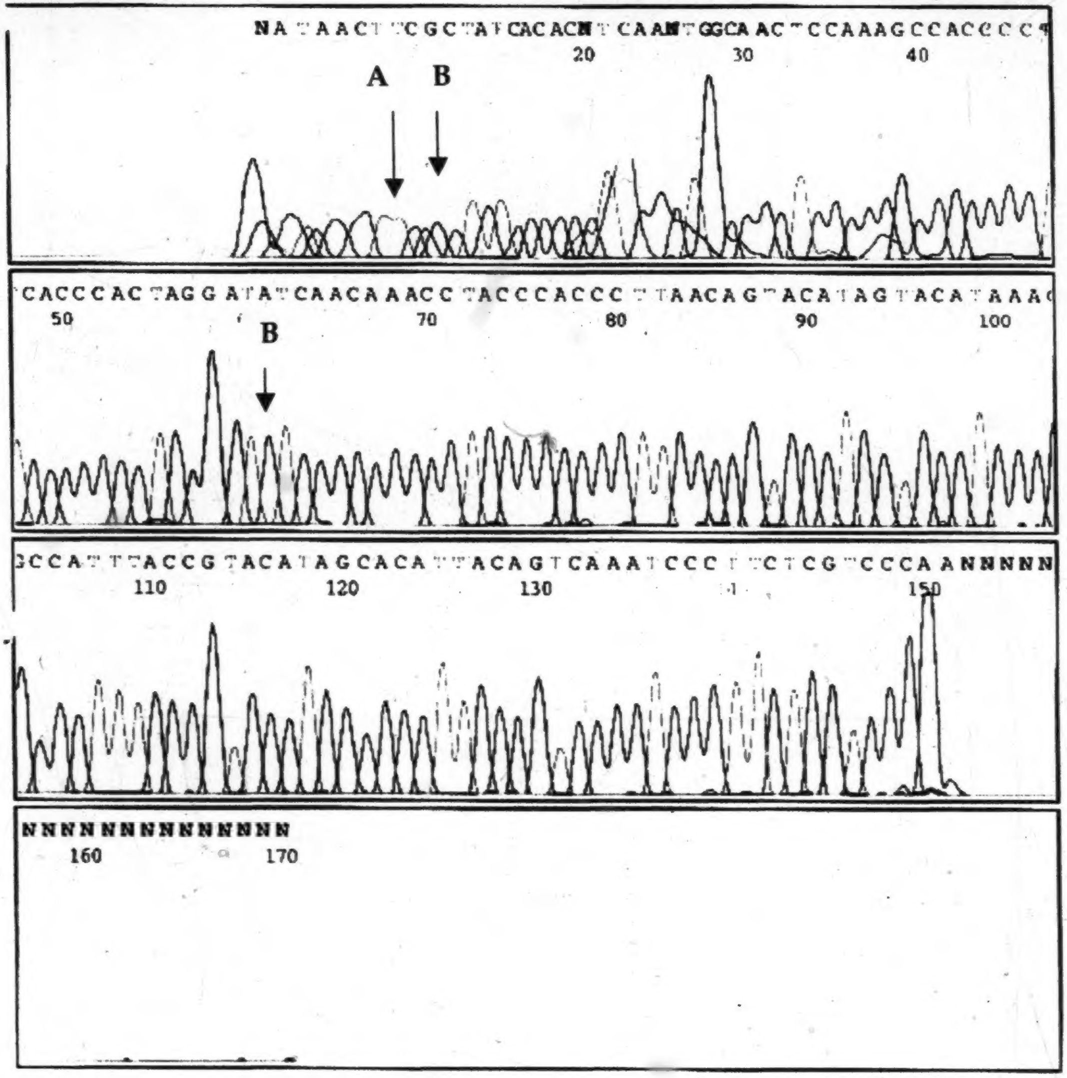
ABI PRISM

Model 377
Version 3.4.1
ABI100
Version 3.3.1

37_130A
L16211
130A
Lane 37

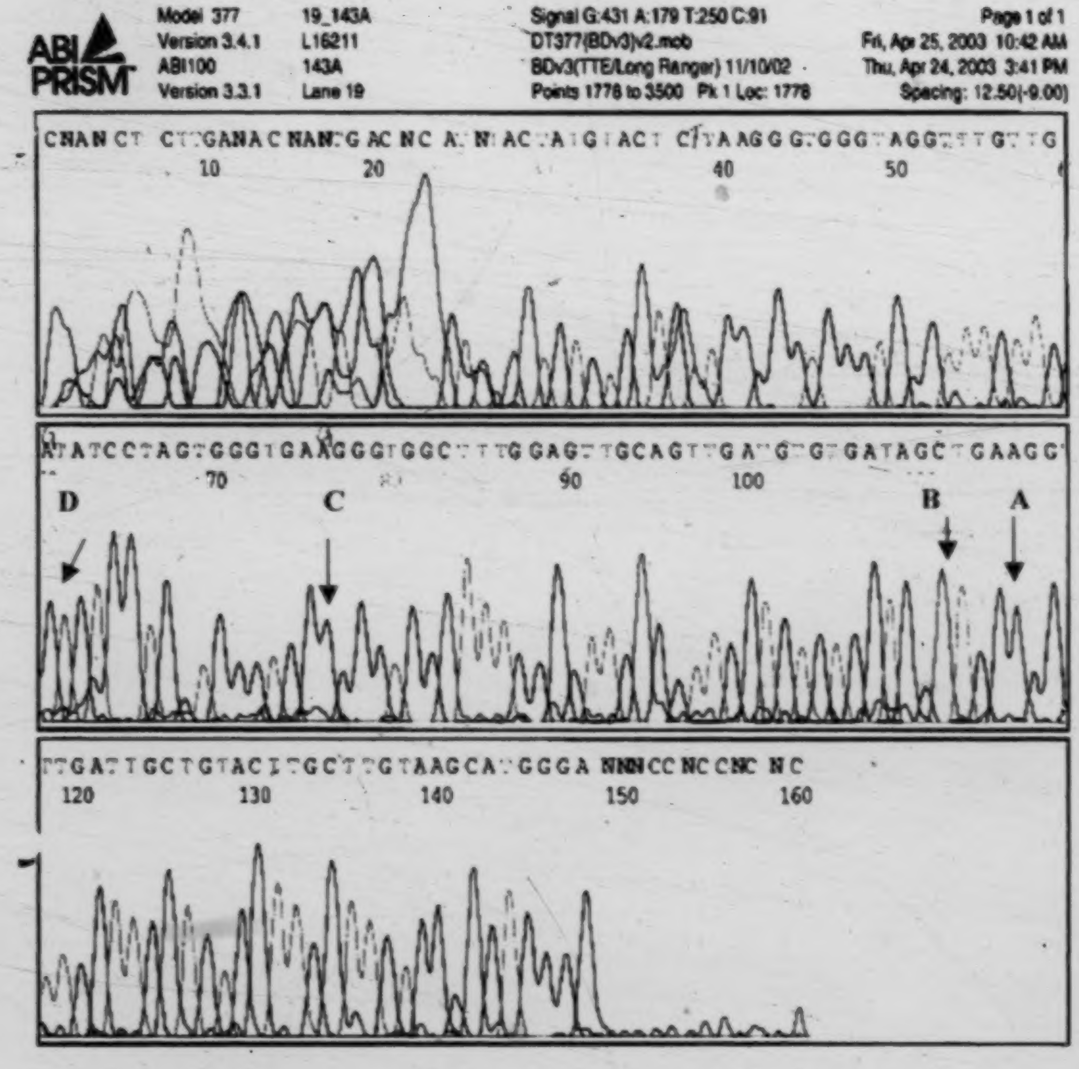
Signal G:190 A:501 T:327 C:355
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1700 to 3800 Pk 1 Loc: 1700

Page 1 of 1
Fri, Jan 31, 2003 12:51 PM
Thu, Jan 30, 2003 5:43 PM
Spacing: 12.50[11.75]



A	**16223**	**T**
B	**16227**	**C**
C	**16278**	**T**

95–143 (Lokomotiv): Haplogroup G2a variant 2 H strand



ABI PRISM

Model 377 | 19_143A | Signal G:431 A:179 T:250 C:91 | Page 1 of 1

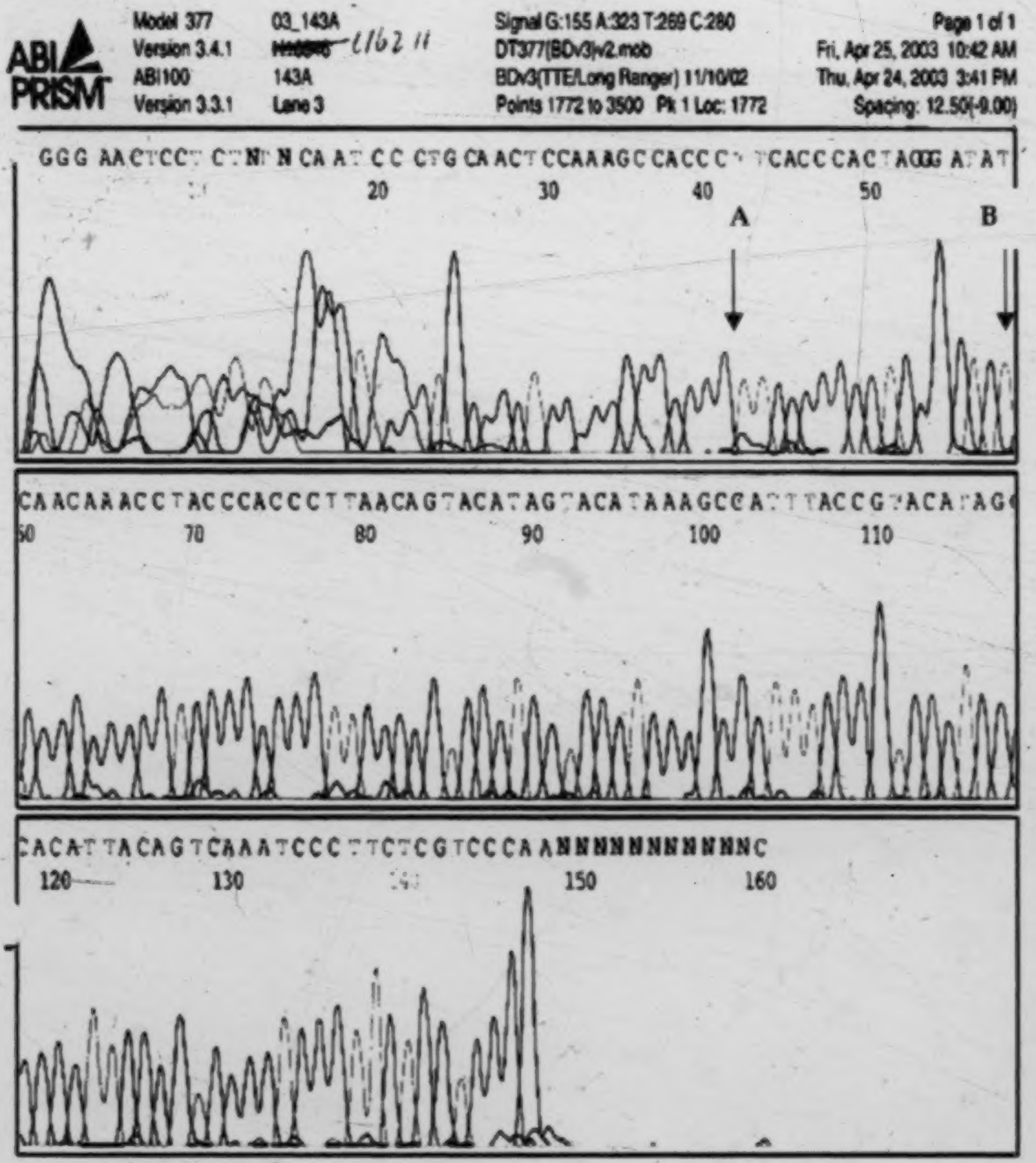
Version 3.4.1 | L16211 | DT377(BDv3)v2.mob | Fri, Apr 25, 2003 10:42 AM

ABI100 | 143A | BDv3(TTE/Long Ranger) 11/10/02 | Thu, Apr 24, 2003 3:41 PM

Version 3.3.1 | Lane 19 | Points 1778 to 3500 Pk 1 Loc: 1778 | Spacing: 12.50(-9.00)

A	16223	T
B	16227	G
C	16262	T
D	16278	T

95–143 (Ust-Ida) Haplogroup G2a variant 2 L strand



Model 377
Version 3.4.1
ABI100
Version 3.3.1

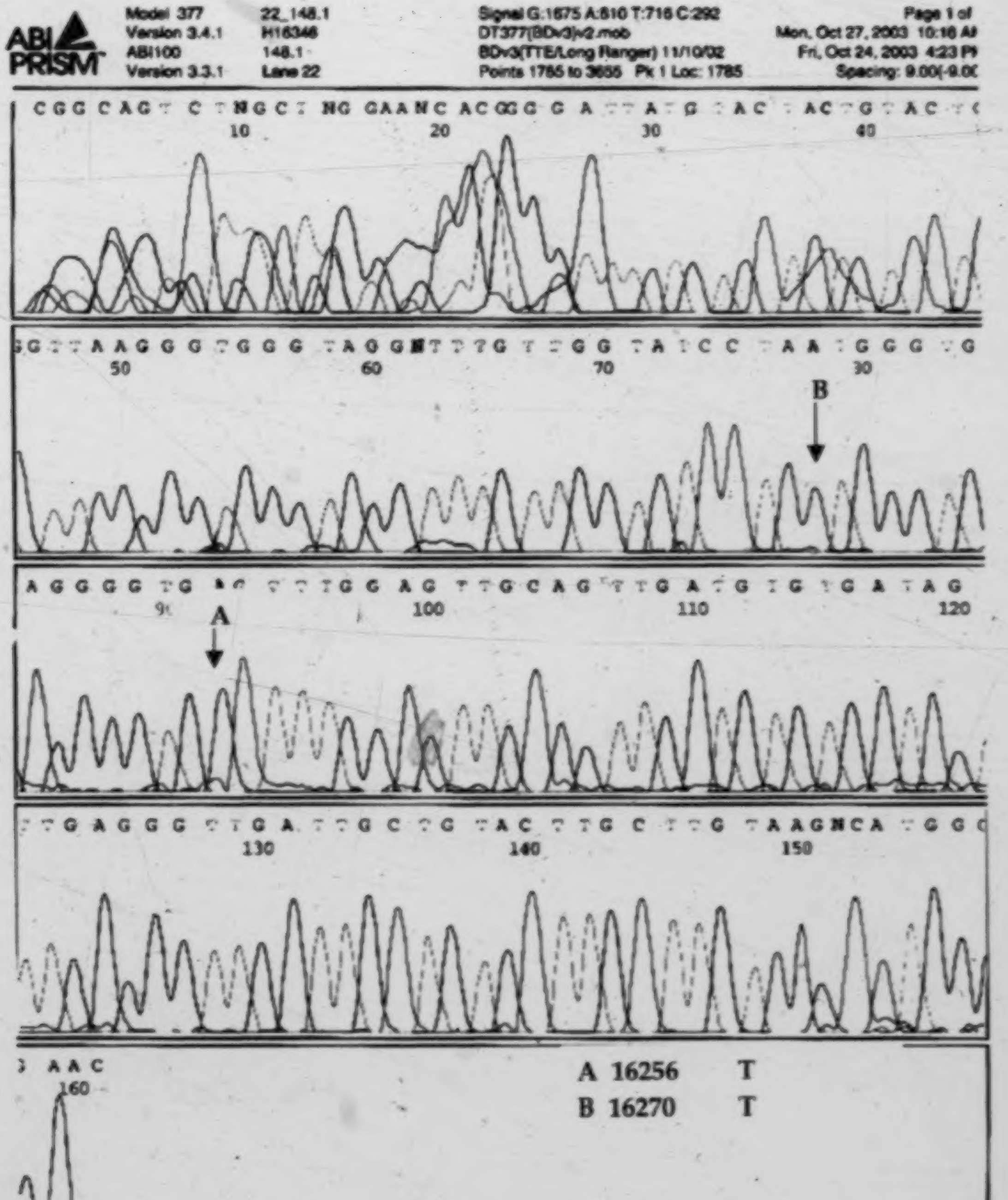
03_143A
[illegible] 16211
143A
Lane 3

Signal G:155 A:323 T:269 C:280
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1772 to 3500 Pk 1 Loc: 1772

Page 1 of 1
Fri, Apr 25, 2003 10:42 AM
Thu, Apr 24, 2003 3:41 PM
Spacing: 12.50(-9.00)

A	**16262**	**T**
B	**16278**	**T**

95–148 (Ust-Ida): Haplogroup U5a H strand



ABI PRISM

Model 377
Version 3.4.1
ABI100
Version 3.3.1

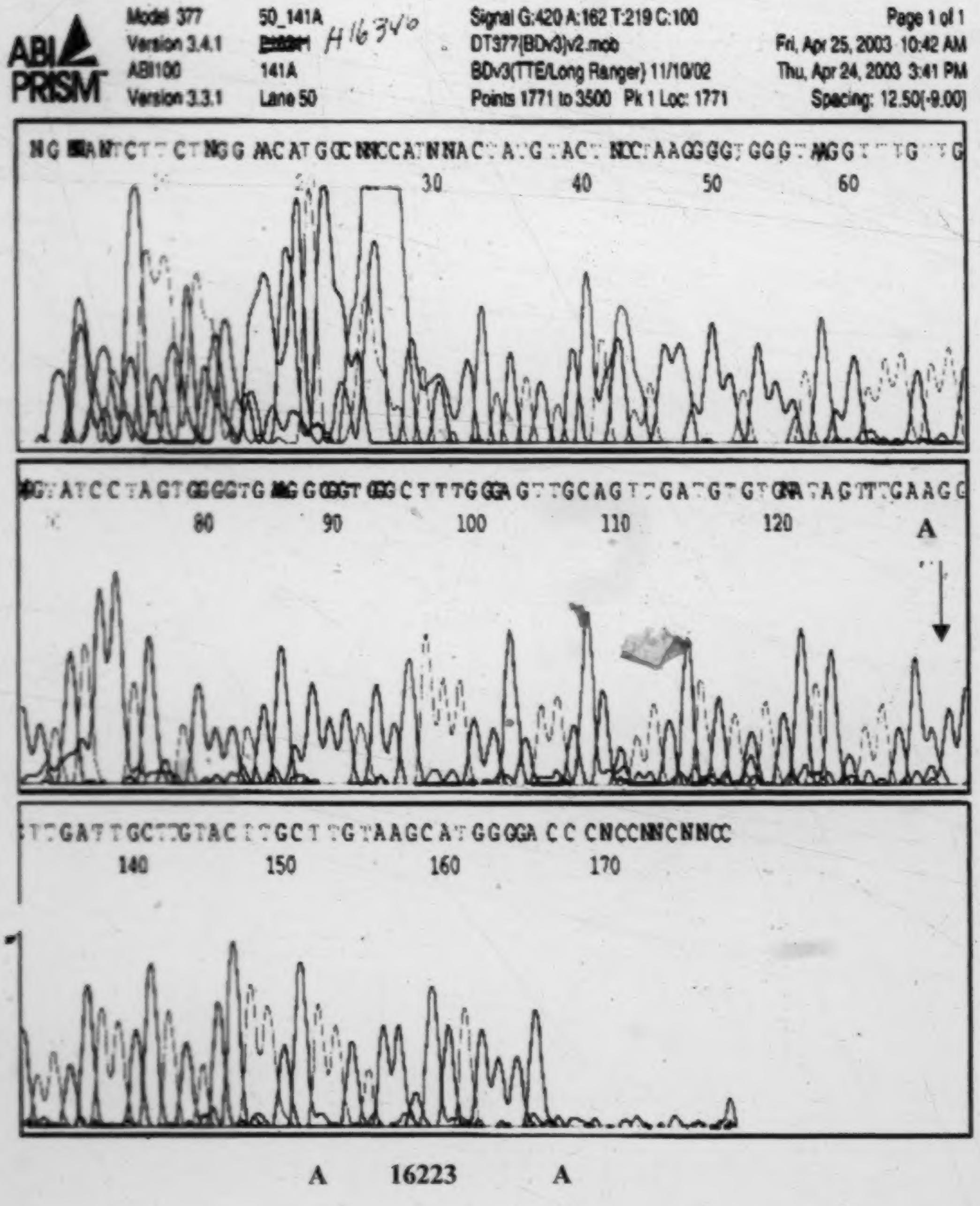
22_148.1
H16346
148.1
Lane 22

Signal G:1675 A:810 T:716 C:292
DT377(BDv3)v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1785 to 3655 Pk 1 Loc: 1785

Page 1 of
Mon, Oct 27, 2003 10:16 A
Fri, Oct 24, 2003 4:23 P
Spacing: 9.00(-9.0

A 16256 T
B 16270 T

95–141 (Ust-Ida) Haplogroup 'Other' Variant 1 H strand



ABI PRISM

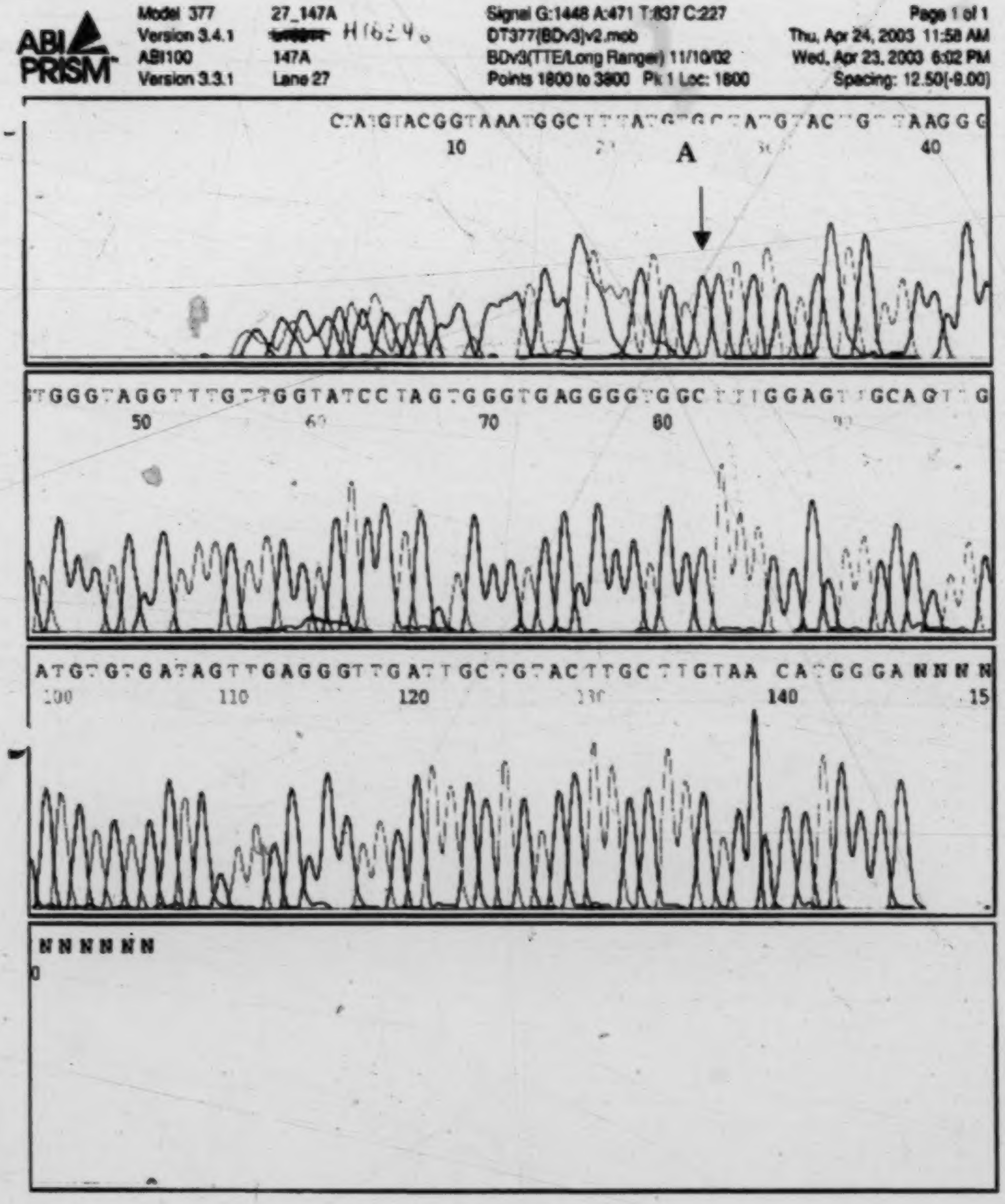
Model 377
Version 3.4.1
ABI100
Version 3.3.1

50_141A
[redacted] H16340
141A
Lane 50

Signal G:420 A:162 T:219 C:100
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1771 to 3500 Pk 1 Loc: 1771

Page 1 of 1
Fri, Apr 25, 2003 10:42 AM
Thu, Apr 24, 2003 3:41 PM
Spacing: 12.50(-9.00)

95–147 (Ust-Ida): Haplogroup 'Other' variant 2: H strand



Model 377
Version 3.4.1
ABI100
Version 3.3.1

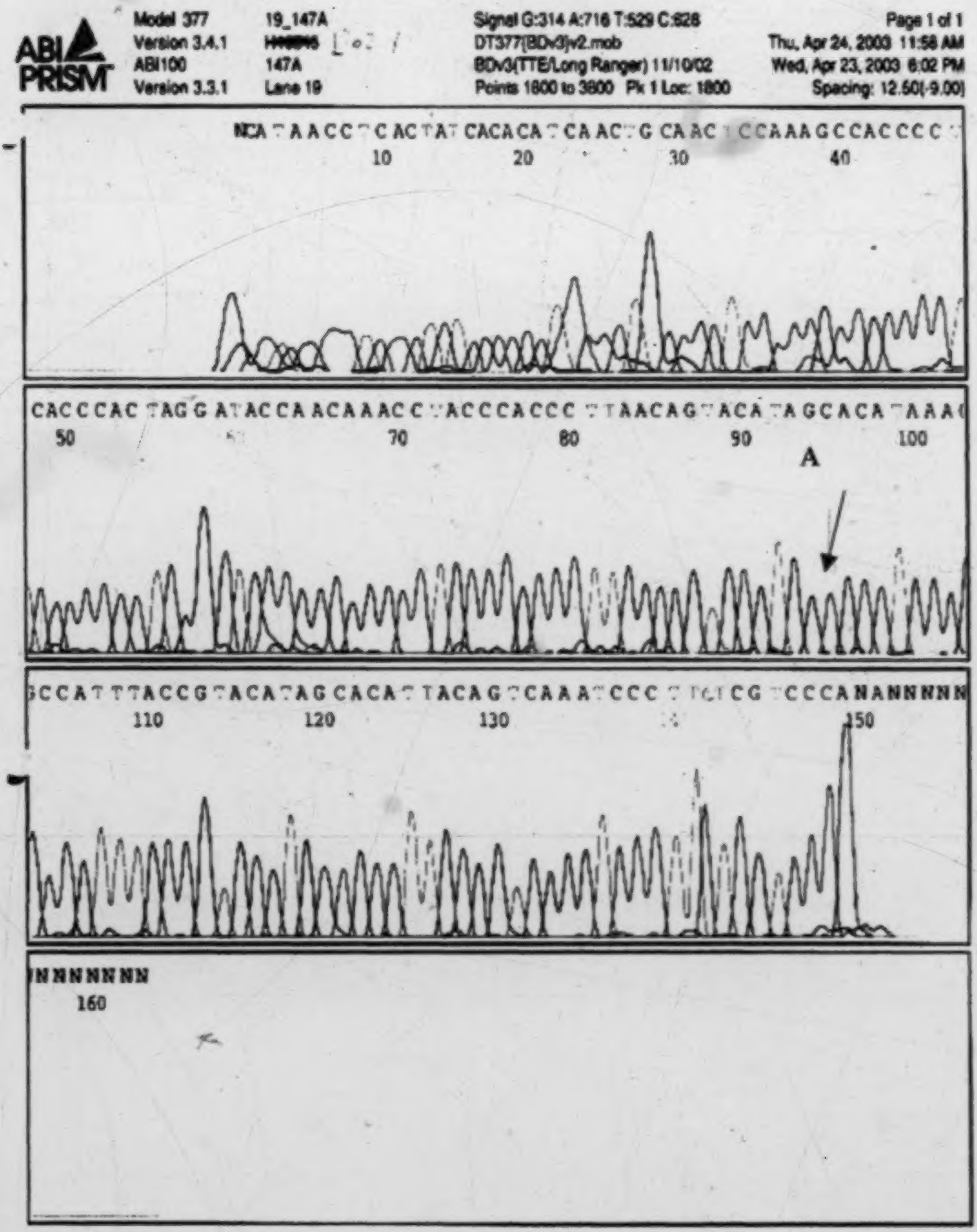
27_147A
[redacted] H16246
147A
Lane 27

Signal G:1448 A:471 T:837 C:227
DT377(BDv3)v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Thu, Apr 24, 2003 11:58 AM
Wed, Apr 23, 2003 6:02 PM
Spacing: 12.50(-9.00)

A 16311 C

95–147 (Ust-Ida): Haplogroup 'Other' variant 2: L strand



ABI PRISM

Model 377
Version 3.4.1
ABI100
Version 3.3.1

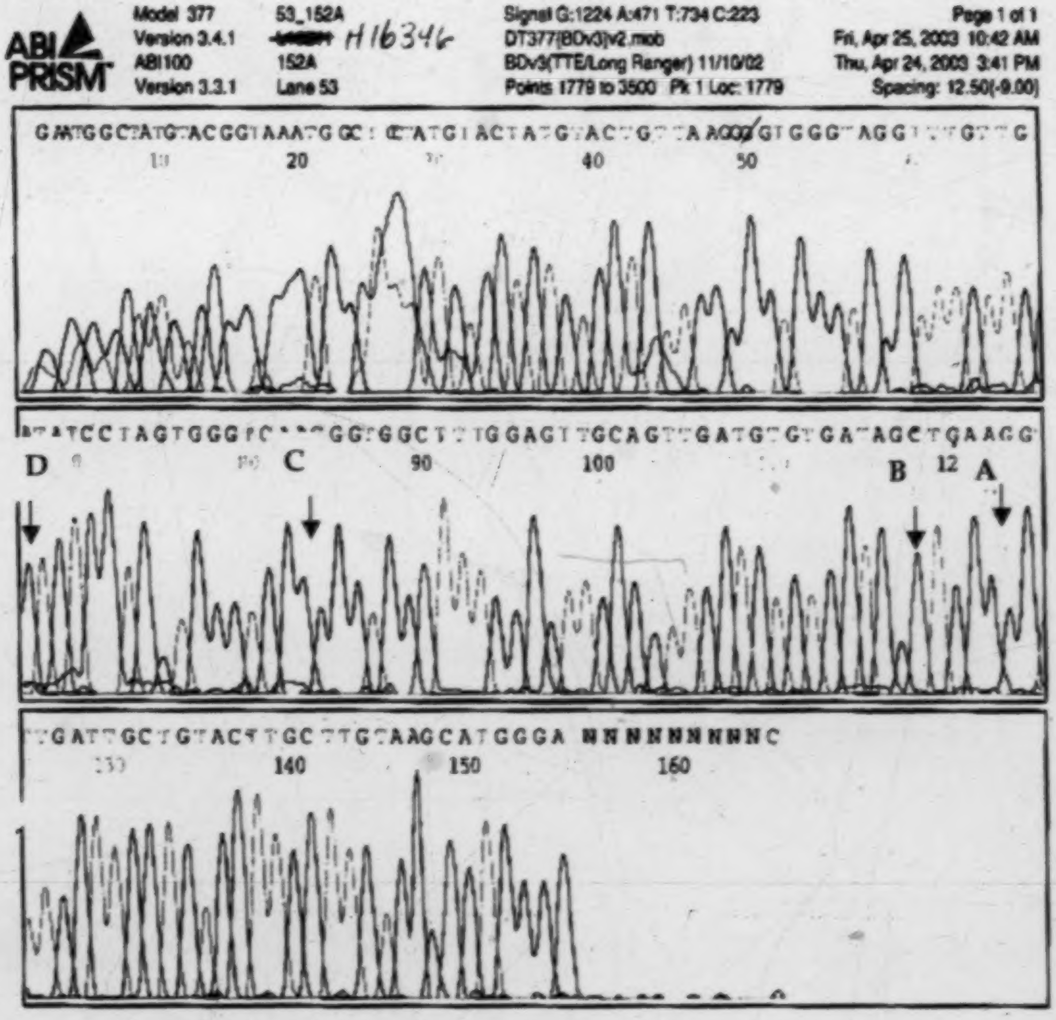
19_147A
[redacted] [o: /
147A
Lane 19

Signal G:314 A:716 T:529 C:628
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1800 to 3800 Pk 1 Loc: 1800

Page 1 of 1
Thu, Apr 24, 2003 11:58 AM
Wed, Apr 23, 2003 6:02 PM
Spacing: 12.50(-9.00)

A 16311 C

95–152 (Ust-Ida): Haplogroup 'Other' sequence variant 4 H strand



ABI PRISM

Model 377
Version 3.4.1
ABI100
Version 3.3.1

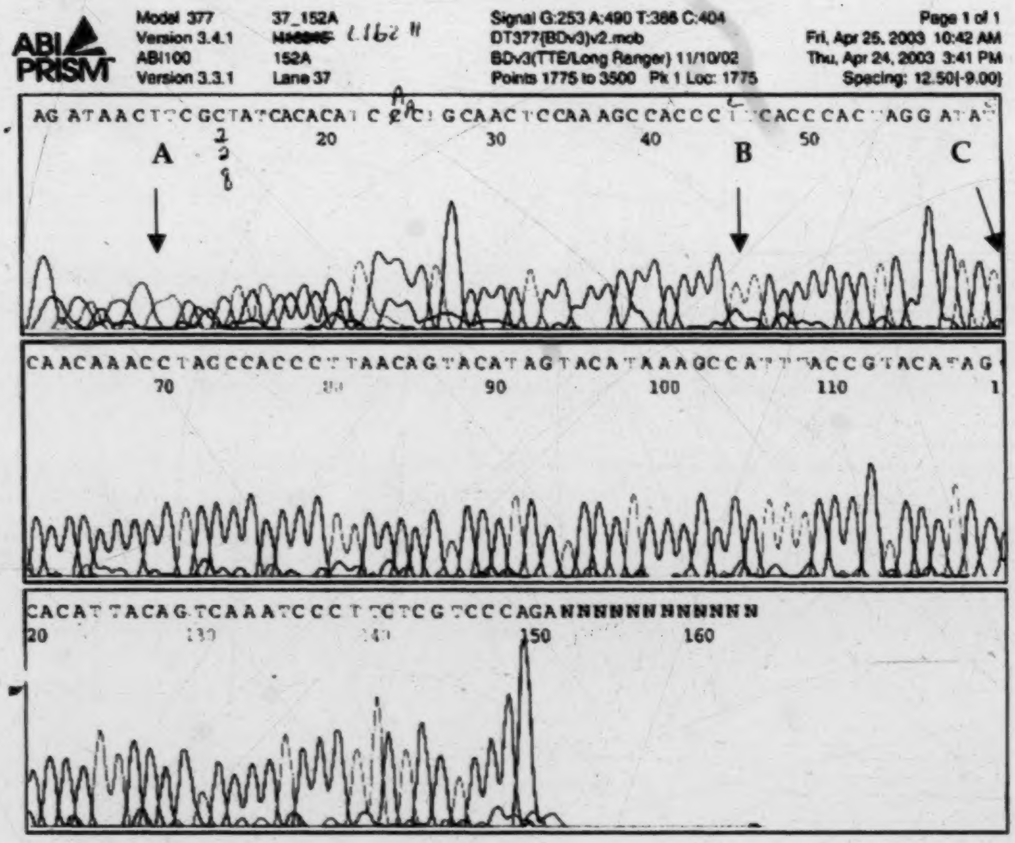
53_152A
[redacted] H16346
152A
Lane 53

Signal G:1224 A:471 T:734 C:223
DT377{BDv3}v2.mob
BDv3(TTE/Long Ranger) 11/10/02
Points 1779 to 3500 Pk 1 Loc: 1779

Page 1 of 1
Fri, Apr 25, 2003 10:42 AM
Thu, Apr 24, 2003 3:41 PM
Spacing: 12.50(-9.00)

A	16223	T
B	16227	G
C	16262	T
D	16278	T

95–152 (Ust-Ida): Haplogroup 'Other' sequence variant 4 L strand



Model 377
Version 3.4.1
ABI100
Version 3.3.1

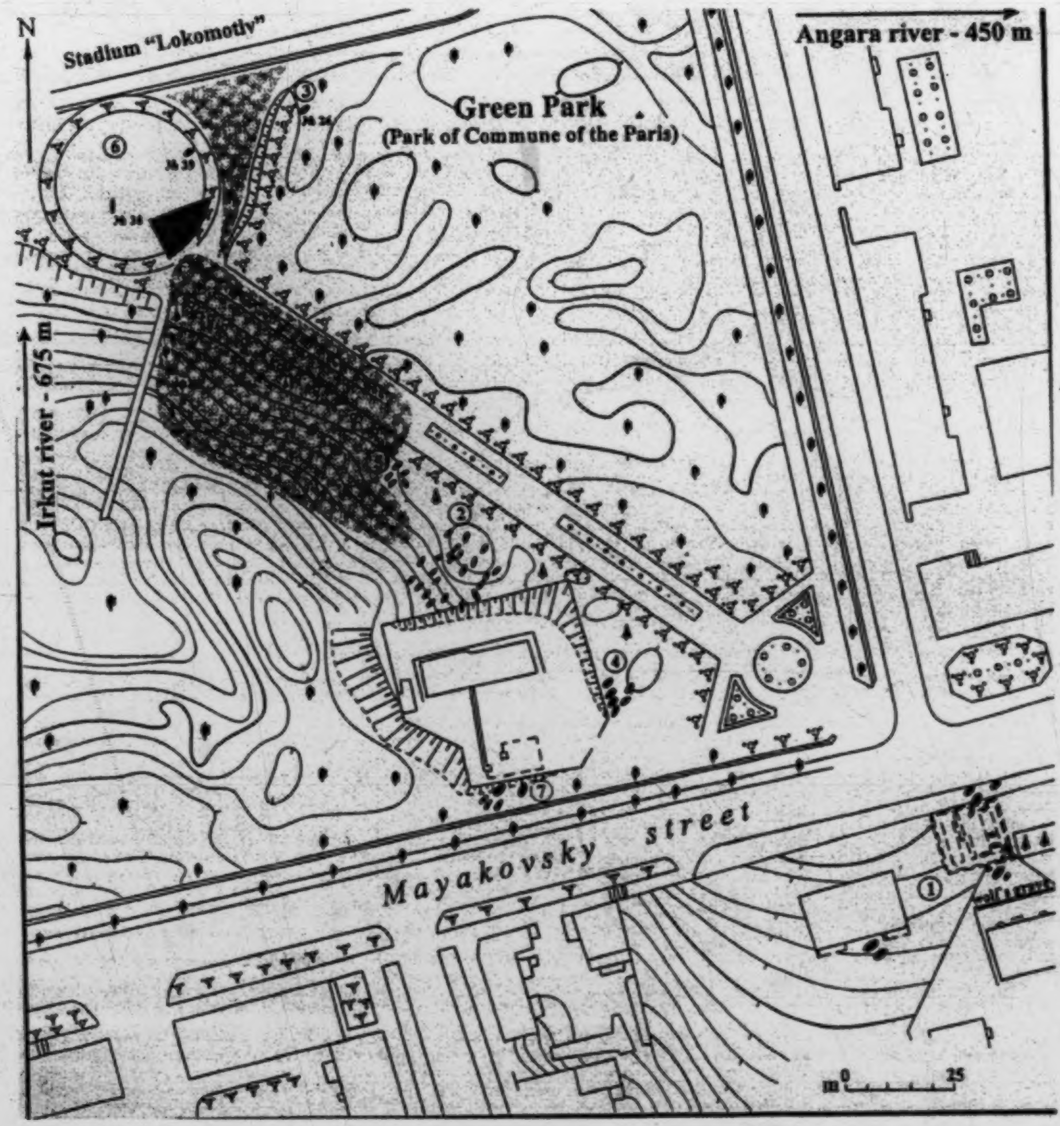
37_152A
~~H46885~~ L162"
152A
Lane 37

Signal G:253 A:490 T:386 C:404
DT377{BDv3}v2.mob
BDv3{TTE/Long Ranger} 11/10/02
Points 1775 to 3500 Pk 1 Loc: 1775

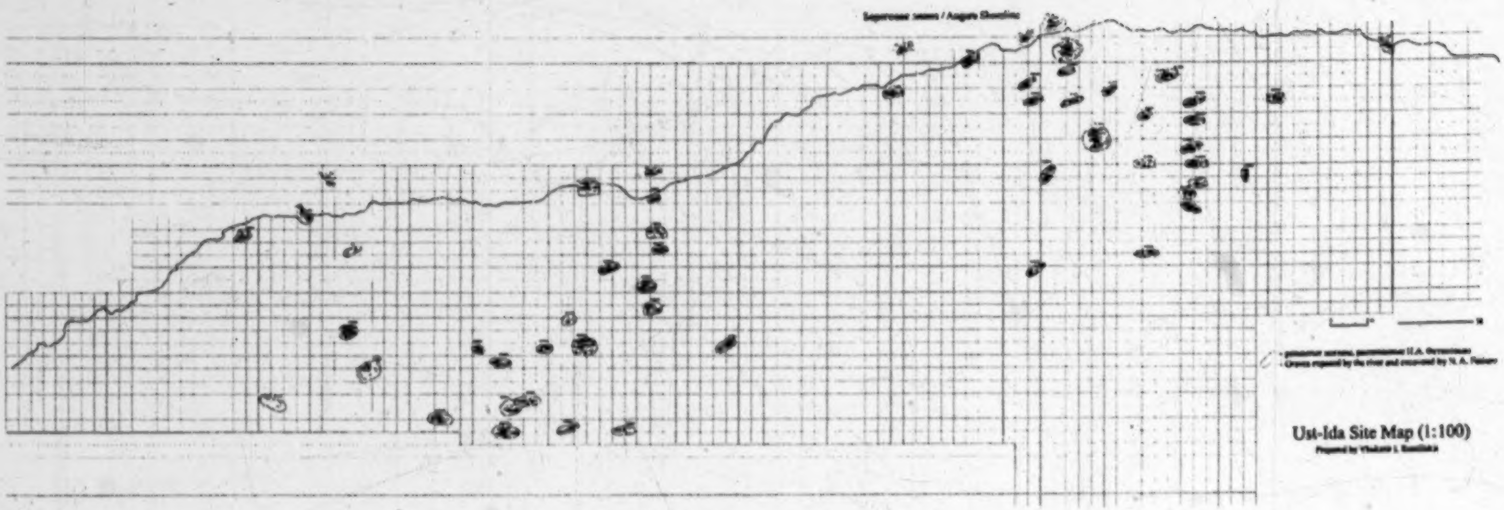
Page 1 of 1
Fri, Apr 25, 2003 10:42 AM
Thu, Apr 24, 2003 3:41 PM
Spacing: 12.50{-9.00}

A	16223	T
B	16262	T
C	16278	T

Appendix 6: Lokomotiv site map

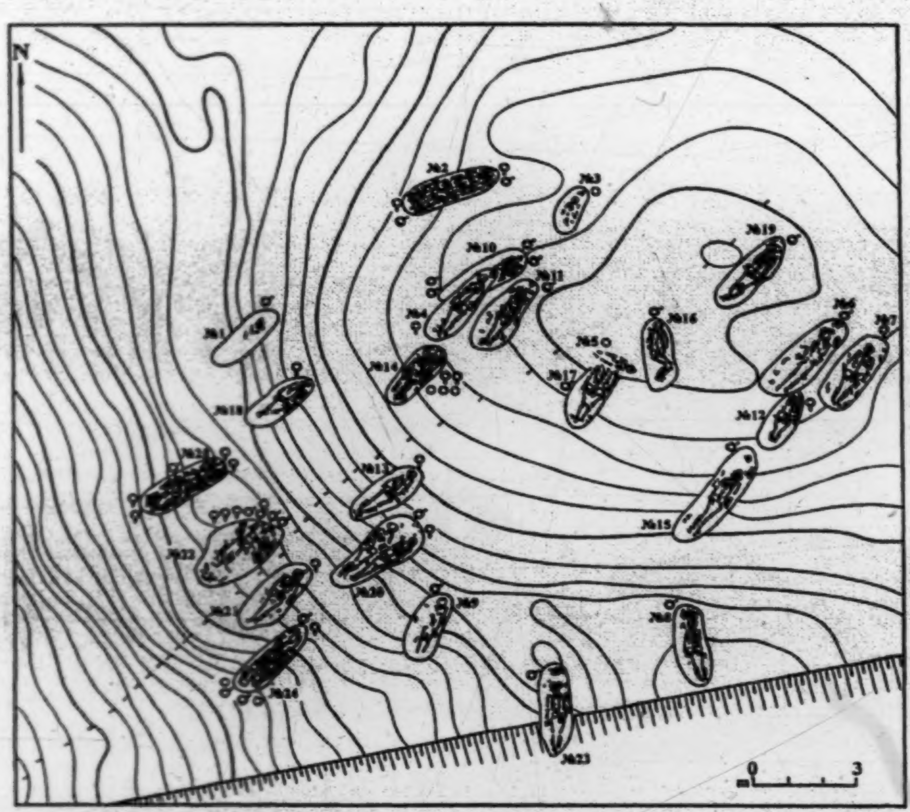

N
Stadium "Lokomotiv"
Angara river - 450 m
Green Park
(Park of Commune of the Paris)
Irkut river - 675 m
⑥
③
②
④
⑦
①
Mayakovsky street
m 0 25

Appendix 7: Ust-Ida site map



Ust-Ida Site Map (1:100)

Appendix 8: Map of Sector 2 burial distribution


N
№2
№3
№19
№10
№11
№4
№16
№5
№6
№7
№1
№14
№17
№18
№12
№13
№15
№22
№20
№9
№8
№21
№24
№23
0
3
m

♂ - male
♀- female
○ - inf
② - Graves L №№ 1 - 25

②

Appendix 9: Molecular sex results

Amelogenin PCR (Mannucci *et al.*, 1994)

Target: 112 bp fragment of exon 1 on amelogenin locus

Primers: Amel1 5′–CCCTGGGCTCTGTAAAGAATAGTG
Amel2 5′–ATCAGAGCTTAAACTGGGAAGCTG

Reaction Conditions

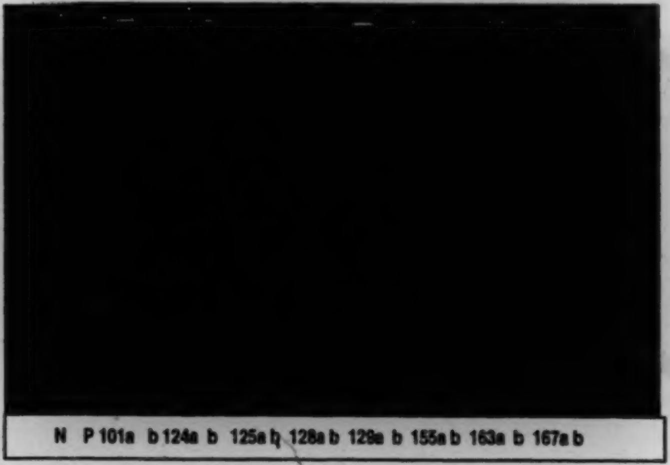
PE480/2400	40 cycles	94 °C	30s	60°C	60s	72°C	30s
MJ PTC	65 cycles	94°C	60s	50°C	60s	72 °C	60s (Vernesi *et al.*, 1999)

*final extension step

Key:

N=	negative PCR control
P=	positive male control
XY	101b; 155a,b;
XX	124a; 128b; 129b
NI	101a; 124b; 125a,b; 128a; 129b; 163a,b; 167a,b

Interpretation: 106/112 bp bands generated for males
106 bp band generated for females



Lokomotiv and Ust-Ida Molecular Sex Data

Notes: **Morph = Morphological Sex**
n Amp = number of PCR amplifications

Lokomotiv

		Amelogenin						
Sample	Grave#	106/112	106/106	112	SRY	Mol. sex	Morph*	n Amp*
95–099	4–1–1	II			pos	XY	F	1
95–104	10–3–1	II		I		XY	M	3
95–106	12–1–1	III				XY	M	3
95–107	13–1–1	II				XY	M	2
95–108	14–3–1			III		XY	imm.	9
95–109	14–4–4	II	III	I		XY	M	3
95–111	16–1–1				pos	XY	M	2
95–112	17–1–1	III		I		XY	M	2
95–114	22–1–5	I		III		XY	M	2
95–115	24–1–1	I	III	I		XY	imm.	7
95–118	27–1–1		I		pos	XY	M	9
95–122	31–2–1	I	II	I		XY	M	4
95–124	36–1–1		III			XX	F	3
95–125	37–1–1	I	III			XX	F	6
95–126	38–1–1	I	I	I		XY	amb	4
95–128	39–1–1		IIII	I		XX	F	6
95–129	41–1–1		III			XX	F	3
95–131	44–1–1	I	III			XY	M	2
95–132	44–2–1	II			pos	XY	M	4

Ust-Ida

		Amelogenin				Mol.		
Sample	Grave#	106/112	106/106	112	SRY	sex	Morph.	N Amp
95–141	5–1–1	I	II	I		XY	imm.	6
95–142	9–1–1		II	II		XY	imm.	2
95–144	12–1–1	I		I	pos	XY	M	5
95–146	16–1–1	II				XY	M	1
95–147	16–2–1	II		I		XY	M	2
95–148	18–1–1		III			XX	imm.	6
95–150	20–1–1	II		I		XY	M	2
95–152	22–1–1		III			XX	F	5
95–153	24–1–1	I	III			XX	F	5
95–154	26–1–1	I		III		XY	M	3
95–155	26–4–1	III		I		XY	imm.	2
95–156	26–5–1		IIII			XX	imm.	3
95–158	30–1–1				pos	XY	F	4
95–159	31–1–1	I	I	I		XY	imm.	2
95–163	36–1–2	I	III			XX	F	5
95–164	38–1–1	III		II		XY	M	4
95–165	40–1–1		II			XX	F	3
95–167	42–1–1	II	I	I		XY	F	2
95–168	43–1–1	I	III	I		XY	M	2
95–169	44–1–1		III			XX	imm.	3
95–170	44–2–1		III			XX	imm.	3
95–171	44–3–1	I	I	II		XY	imm.	6
95–174	48–1–1	II				XY	M	1
95–177	53–2–1	II	I	I	pos	XY	imm.	8
95–179	55–1–1	II				XY	M	2
95–180	55–2–1	II				XY	M	2

Appendix 10: *Curriculum vitae*

Karen P. Mooder

Education

Present University of Alberta Edmonton, AB

Ph.D. Candidate, Department of Laboratory Medicine and Pathology

- Dissertation entitled "Neolithic Polymorphisms and the Lives of Siberian Hunter-Gatherers" (defence anticipated March 2004).
- Candidacy exam completed August 2001
- Key Coursework: DNA Systematics , Forensic Anthropology, Human Genetics, Human Osteoarchaeology, Human Osteology, Mortuary Archaeology
- GPA: 8.2 of 9

1997 University of Alberta Edmonton, AB

Bachelor of Science in Medical Laboratory Science (Honours)

- First Class Standing in fourth year
- Undergraduate Thesis entitled "Optimizing the Analysis of Mitochondrial DNA from Suboptimal Samples"

1991 Northern Alberta Institute of Technology Edmonton, AB

Diploma in Medical Laboratory Technology

- CSMLS certification #39049 obtained 1991

Publications

Refereed Publications

- Mooder K, Schurr TG, Bamforth F, Bazaliiskii V (2003). Mitochondrial DNA and Archaeology: The Genetic Characterisation of Prehistoric Siberian Hunter–Gatherers. *Northern Hunter Gatherers–Research Series*, CCI Press, Edmonton pp. 193–202

Manuscripts in Preparation

- Mooder K, Hicks M, Bamforth F. Verifying The Authenticity of mtDNA Sequence Data from Human Skeletal Remains.

- Mooder K, Schurr TG, Bamforth F, Bazaliiskii V. Population Affinities of Neolithic Siberians: a Snapshot from Prehistoric Lake Baikal.

- Mooder K, Schurr TG, Bamforth F, Bazaliiskii V. A Tale of Two Cemeteries: Inferring Kinship and Social Relations from Ancient DNA.

Invited Exhibitions

- Mooder K (2003). DNA and Human Identification. National Medical Laboratory Week, Calgary Laboratory Services, April 24, Calgary, AB.
- Mooder K (2002). "If graves could talk, what secrets they would tell". Media Interview, CBC Radio One, December 27, Edmonton, AB.
- Mooder K (2001). DNA and Human Remains: The Marriage of Biochemistry and Anthropology. Alberta Society of Clinical Chemists fall meeting, October 14, Jasper, AB.

Conferences

- Mooder K, Hicks M, Bamforth F (2003). Verifying the Authenticity of Ancient DNA. International Association of Legal Medicine XIX Congress, September 3–6, Milan, IT (talk).
- Mooder K, Schurr T, Bamforth F, Bazaliiski V (2003). Extracting Kinship and Social Relationships from Genetic Data: A study of Prehistoric Cis-Baikal Mortuary Populations. Canadian Archaeological Association annual meeting, May 7–10, Hamilton, ON (talk).
- Drouin B, Lieverse A, McKenzie H, Mooder K, Robertson C, Weitzel M, White D (2003). Graduate Student Contributions to the Baikal Archaeology Project. Northern Research Days. January 17, University of Alberta (poster).
- Mooder K and Bamforth F (2002). Neolithic Polymorphisms and the Lives of Prehistoric Siberian Hunter–Gatherers. Human Origins and Disease Symposium, October 30–November 3, Cold Spring Harbour, NY (talk).
- Mooder K and Bamforth F (2002). Sex Identification of Human Skeletal Remains. Macgregor Research Day, Department of Laboratory Medicine and Pathology, September 26, Edmonton, AB (talk).
- Mooder K and Bamforth F (2001). Mitochondrial DNA and Archaeology: The Genetic Characterisation of Prehistoric Siberian Hunter–Gatherers. Canadian Archaeological Association annual meeting, May 6–9, Banff, AB (talk).
- Mooder K and Bamforth F (2000). Mitochondrial DNA and Archaeology: Genetic Analysis of Skeletal Remains from Neolithic Siberia. Medical Genetics in Alberta Day, June 6, Red Deer, AB (talk).
- Mooder, K and Bamforth F (2000). Ancient DNA analysis of Skeletal Remains from Neolithic Siberia. Anthropology in the 21st Century: Beyond the Ivory Tower, February 18–20, Edmonton, AB (talk).

Grants and Awards

- Graduate Student Teaching Award, Faculty of Medicine and Dentistry (2003)
- Graduate Student Presentation Award, Macgregor Research Day (2002)
- Circumpolar/Boreal Alberta Research (C/BAR) Grant Award (2000-2003)
- Northern Scientific Training Program (NSTP) Grant Award (2000-2001)
- HE Bell Scholarship (1998)

Professional Experience

2000 to 2003 University of Alberta Edmonton, AB

Sessional Instructor, Division of Medical Laboratory Science

- Responsible for the development and delivery of laboratory curriculum for Clinical Chemistry courses for phase I Medical Laboratory Science students.
- Lecturer in senior undergraduate courses in Molecular Genetics.
- Participation in departmental administration through involvement on academic admissions committee and program accreditation.

1998 to 1999 University of Alberta Edmonton, AB

Research Assistantship, Department of Laboratory Medicine and Pathology

- Performed DNA analysis for the purpose of human identification in collaboration with the Department of Anthropology and the RCMP.

1997 to 1998 University of Alberta Edmonton, AB

Research Technologist, Department of Medical Microbiology and Immunology

- Primarily responsible for Southern Blot analysis of *Mycobacterium tuberculosis* from clinical isolates as part of a national epidemiological study.
- Provided technical support for studies involving the isolation *of M. tuberculosis* from animal models.
- Provided training in molecular biology techniques for undergraduate students and visiting professionals.

1991 to 1995 Northern Lights Regional Health Centre Ft. McMurray, AB

Medical Laboratory Technologist I

Responsible for the accurate analysis and reporting of patient samples in Immunohematology, Hematology and Chemistry on a 24 hour, seven day rotation.

Professional Affiliations

Hum Biol Association, Canadian Federation of University Women.